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DEVELOPING BROAD-SPECTRUM RESISTANCE TO ANTHRACNOSE IN COMMON BEAN THROUGH GENE PYRAMIDING

By

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A thesis submitted to the Directorate of Research and Graduate Training for the award of the degree of Doctor of Philosophy of Makerere University

August 2017

DECLARATION

This thesis is entirely my own work and has not been presented for a degree award in any University

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DEDICATION

To my family Elisha, Josiah, Karen and Rachael

ACKNOWLEDGEMENT

I express my sincere gratefulness to the following people and organizations who contributed in various ways to make this work a success;

My graduate advisors and supervisors; Prof. Phinehas Tukamuhabwa and Dr. Herbert Talwana of the School of Agricultural Sciences, Makerere University; Dr. Nkalubo Stanley and Dr. Pamela Paparu of the Legumes Program, NaCRRI and Dr. Mukankusi Clare of CIAT-Uganda, for advising and guiding me until this work became a success. I specially thank Professor Patrick Rubaihayo for the critical supervision and guidance during write-up of this thesis.

Field research and Laboratory technical staff, Vincent Kyaligonza and Tony Mukwaya at Biosciences lab facility, NaCRRI, Allan Male, Kiviiri Samuel and Cathy Acam at the CIAT lab facility, NARL, for their technical support in laboratory based research work; Sheikh Ssebuliba, Naluwooza, Arafat, Musoke Steven and Khasifa of CIAT-Uganda, NARL and Nakimera Juliet and Lydia of NaCRRI for all the field and screen house support.

Fellow students undertaking different aspects of beans research namely Aston Ebinu, Ongom Patrick, Esther Arengo, Dennis Ocii, Olango Nicholas for their continual encouragement and sharing of experiences. Fellow students in the first cohort of the PhD program in Plant Breeding and Biotechnology such as Kawube Geoffrey, Jushua Mudingoto, Gabriel Ddamulira, Pariyo Anthony, Sadiq Kassim, Wasukira Arthur, Laban Turyagyenda, Bombom Alexander, Wagaba Henry, Abel Sefasi, Maphosa Mcbesi, Langa Tembo to mention but a few

The Uganda Council of Science and Technology (UNCST) MSI project, the National Agricultural Research Organization (NARO) ATAAS project and CIAT/ PABRA for funding my tuition and this research work.

Prof. Adipala Ekwamu, Dr. Patrick Okori, Dr. Richard Edema, Prof. and Mrs. Gibson, for conceiving, delivering the vision of a PhD in Plant Breeding and Biotechnology at Makerere University and for their constant parental encouragement and guidance.

The National Agricultural Research Organization (NARO) training committee, Directorate of Human Resource for supervisory and financial support; and particularly Dr. Julius Mukalazi

and Dr. Sadik Kassim, Directors of Research at Abi ZARDI where I have been serving as a Research Officer during my PhD studies.

My family; Rachael my wife and children Karen, Emma and Josiah for your patience and understanding during the difficult times and for surrendering their right to have all my attention during the period of this work

The good LORD who gave me patience and endurance through it all.

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GENERAL ABSTRACT

Bean anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. et. Magn) Lams. Scrib., is one of the most widespread and economically important fungal diseases of the common bean. The pathogen possesses a high genetic and pathogenic variability, which causes it to overcome single-gene resistance in cultivars. Pyramiding of resistance genes in commercial varieties is a strategy that would ensure a more effective resistance and cause reduction of yield losses. This study was conducted with the overall objective to contribute to the understanding of gene pyramiding for broad-spectrum resistance to bean anthracnose disease in Uganda. The specific objectives were; a) To determine the pathogenic variability of *Colletotrichum lindemuthianum* in Uganda; b) To evaluate effectiveness of pyramided resistance genes against bean anthracnose disease; and c) To determine the genetic variability and relationship between number of pyramided resistance genes on yield traits among advanced common bean populations.

To determine pathogenic variation of *C. lindemuthianum*, samples of common bean tissues with anthracnose symptoms were collected in eight districts namely Kabarole, Sironko, Mbale, Oyam, Lira, Kapchorwa, Maracha and Kisoro and 51 isolates which sporulated successfully on Potato dextrose Agar and Mathur's media were used to inoculate 12 common bean standard differential cultivars under controlled conditions. Five plants per cultivar were inoculated with each isolate and evaluated for their reaction using a 1 – 5 severity scale (Inglis *et al*., 1988). Races were classified using the binary nomenclature system proposed by Pastor Corrales (1991). Sequence Characterized Amplified Region (SCAR) markers were used to facilitate the process of pyramiding and tracking three anthracnose (*Co-4*^{2}/ *Co-4*^{3}, *Co-5* and *Co-9*) resistance genes using a cascading pedigree pyramiding scheme. Detached leaf trifoliates of plants from 40 $F_{4:6}$ families were screened under controlled conditions with four *C. lindemuthianum* races and severity scored using a 1 – 9 severity scale (Balardin *et al*., 1997). 53 $F_{4:5}$ and 69 $F_{4:6}$ families were evaluated in the field for yield traits performance. Disease severity and yield traits data were subjected to ANOVA to uncover variability. Correlation and path analysis were done to establish relationships between number of pyramided genes and the yield traits.

ANOVA for disease severity among the standard differential cultivars revealed that cultivar and isolate effects were significant ($P \leq 0.001$). The 51 isolates from eight districts grouped into 27 pathogenic races. Sironko district had the highest number of races followed by Mbale and Kabarole. Races 2047 and 4095 were the most frequent. Race 4095 was the most virulent and

caused a susceptible (S) reaction on all 12 differential cultivars followed by races 2479, 2047 and 2045. Two races, 4094 and 2479, caused a susceptible reaction on the differential cultivar G2333, known to possess a high degree of resistance. The differential cultivars G2333, Cornell 49-242, TU and AB136 showed the most broad-spectrum resistance and are recommended as sources of effective resistance against *C. lindemuthianum* in Uganda.

ANOVA for disease severity among the gene pyramid groups revealed significant differences (*P<0.001*) among races, genotypes and significant Race x Genotype interaction. The five gene pyramid group means were significantly different from each other (*P<0.01*) of which, *Co-4^2+Co-5+Co-9* and *Co-4^2+Co-5* exhibited the lowest mean disease score to all the four races indicating both a high degree and a broad spectrum of resistance. The group *Co-4^3+Co-9* had the highest mean disease severity. The single-gene groups were significantly different from each other (*P<0.01).* The *Co-4^2* and *Co-5* gene groups both conferred resistance to all the four races 352, 713, 767 and 2047, while the *No-gene* (5.9±0.21) group was overcome by all the four races. *Co-4^2* group had the least mean severity across races followed by *Co-5*, Co-*4^3*, *Co-9* and *No-gene*. The single gene *Co-4^2*was not significantly different from the best pyramid groups *Co-4^2+Co-5+Co-9* and *Co-4^2+Co-5* (*P<0.01*) but was better than the other three pyramid groups. Similarly the *Co-5* single gene was not significantly different from *Co-4^2+Co-5, Co-4^2+Co-9* and *Co-5+Co-9* gene pyramid groups (*P<0.01*). The single gene *Co-9* was found to be antagonistic in pyramid combinations and should be avoided in resistance breeding programs. The two single genes *Co-4^2* and *Co-5* showed broad-spectrum resistance to *C. lindemuthianum* and are therefore recommended for combining with other single genes to develop highly effective gene pyramids.

ANOVA for yield associated traits among advanced populations revealed significant variability (*P<0.01 and P<0.05*) for all yield traits and further revealed that Phenotypic Coefficients of Variability (PCV) estimates were higher than the Genotypic Coefficients of Variability (GCV) estimates indicating the importance of environmental effects in the expression and improvement of these traits. Broad sense heritability (h_b^2) and Genetic advance among populations was low for number of pods per plant, number of seeds per plant and seed weight per plant indicating importance of non-additive gene action and implying that selection would not be effective in improving these traits but other breeding strategies such as heterosis breeding would be appropriate for improving the traits. Three $F_{4:6}$ lines with pyramided genes namely 16.3.3.11.160.5.6, 44.1.4.5.142.4 and 16.1.3.8.136.2 were among the best 10 yielders.

However, number of pyramided genes had a significant negative correlation with seed weight per plant (-0.17), number of pods per plant (-0.24, $p<0.05$) and number of seeds per plant (-0.19, $p<0.1$); and path coefficient analysis revealed a significant ($P<0.05$) negative indirect effect of number of pyramided genes on seed weight per plant via number of seeds per plant (-0.25). These findings suggest that pyramiding of higher number of resistance genes may reduce overall yield by negatively affecting some yield trait. The significance of yield foregone may, however, be reduced through appropriate breeding strategies which ensure increased resistance through gene pyramiding and increased yield gain.

CHAPTER ONE

General introduction

1.1 Background

The common bean, *Phaseolus vulgaris* (L.), is a diploid (2n = 22) with a genome size ranging from 450 to 650 mbp for haploid genome (Broughton *et al*., 2003). It was domesticated about 7,000 years ago (Kaplan, 1965) in two centers of Mesoamerica (Mexico and Central America) and the Andean region and was introduced in sub-Saharan Africa in the 16th century (Greenway, 1945) by Portuguese traders (CIAT, 2001). The common bean was introduced to the highlands of Eastern Africa about 400 years ago and the highlands are now a secondary center of genetic diversity (Schwartz and Pastor-Corrales, 1989). Beans are the most important legume for human consumption in the world and they are considered to be the second most important source of dietary protein and the third most important source of calories (FAOSTAT, 2012), for nearly 500 million people in Africa, Latin America and the Caribbean (Cortés et al., 2013). Common bean is characterized as a "nearly perfect" food due to its high protein content and generous amounts of fiber, complex carbohydrates, and other dietary necessities (CIAT, 2001). A single serving of beans provides at least half the United States Department of Agriculture's recommended daily allowance of folic acid (CIAT, 2001) and one of the vitamins in the B complex that is especially important for pregnant women (Peake, 2013). It also supplies 25 to 30% of the recommended levels of Iron (Fe) and meets 25% of the daily requirement of Magnesium (Mg) and Copper (Cu) as well as 15% of the Potassium (K) and Zinc (Zn) (CIAT, 2001). The nutritional balance of beans, therefore, makes it a means of preventing malnutrition especially among the low income class.

In Uganda, beans are the most important source of proteins providing up to 45% of the protein intake for the population (Kilimo Trust, 2012). The National Development Plan (2010), under Section 5.1 on Agricultural development, identified beans among the 10 selected national strategic commodities and called for their accelerated production. Although beans are largely produced for subsistence mainly by women farmers in sub-Saharan Africa (Wortmann *et al.,* 1999), their short maturity period, ease of handling and storability make them a coveted cash crop for small-scale farmers (David, 1999) and, therefore, play an essential role in the

sustainable livelihoods of smallholder farmers, providing both food and income security. Beans are also highly valued by the resource poor because all parts of the plant can be used; the grain is eaten fresh or dried, the leaves are used as vegetables and the stalk is used to make soda ash (David, 1999).

1.2 Bean production

Global bean production in 2013 was approximately 23.4 million metric tons, with 23.2% and 25.8% of the world production in Latin America and Africa, respectively (Murrel, 2016), with an annual market value of about US $10 billion (FAOSTAT, 2012). Brazil is the main producer of common bean followed by India, China, United States of America and Mexico (FAOSTAT, 2008). Tanzania, Uganda and Kenya lead in production on the African continent with volumes estimated at 950,000MT, 455,000MT and 390,598MT respectively in 2010 (Kilimo Trust, 2012). Other important producers in Africa include D.R Congo, Burundi, Rwanda and Ethiopia (Pachico, 1993). Beans are the most important legume crop in Uganda occupying an important niche in the Agricultural sector and farm household economy (Opio *et al*., 2001) and accounting for 6.1% of national agricultural GDP, ranking fifth behind bananas, cassava, beef and milk (FAOSTATS, 2008). By 2010, Ntungamo district was the leading producer with a total of 138,000 MT, followed by Mubende with 78,000 and Amuru district with 75,000 Mt (UBOS and MAAIF, 2010).

1.3 Production constraints

The average global yield of common beans has been observed to be generally declining since the early 2000s (FAOSTAT, 2008). Recorded yields of bush beans frequently reach 3 tons/ha in experimental conditions while national averages throughout the tropics typically range from 500 to 700 kg/ha implying a wide yield gap in relation to yield potential. Uganda's common bean productivity per hectare has also been on the decline since 2001 with recorded yield average of 0.4 – 0.8MT/ha depicting a major shortfall from the potential yield of 1.5 – 2.5MT/ha realized with improved varieties (Kalyebara, 2008). This is attributable to several production constraints such as pests, diseases, declining soil fertility, drought, poor agronomic practices (Beebe, 2012), low adoption and low replacement rates of improved seed (ISSD, 2012).

Abiotic stresses like drought are estimated to affect 60% of bean growing areas worldwide especially tropical lowlands of South America and Africa (Beebe *et al*., 2012) and can maintain average bean yields below 1 ton/ha in the tropics (Wortmann *et al.*, 1998). Soil constraints including low levels of phosphorous (P) and nitrogen (N) and toxicities of aluminum (Al) and manganese (Mn) associated with acid soil and low calcium (Ca) availability are the most widespread limitations and in contrast to drought, are present every year (Thung and Rao, 1999). Low levels of soil organic matter in degraded soils further compounds these problems (Beebe *et al*., 2012).

Diseases and pests are the two most important biotic constraints of common bean production worldwide (Rodriguez and Barnado, 2014). More than 45 diseases are reported to affect the common bean incited by fungi, bacteria, viruses and nematodes (Vieira, 1983). At lower altitudes in the Great Lakes Region, and elsewhere in eastern and southern Africa, insect pests are a significant limiting factor to production. The bean fly *(Ophiomyia spp*.) can cause substantial damage, especially on less fertile land although application of fertilizer on farm may effectively suppress its damage (Shwartz and Pastor-Corrales, 1989).

The major diseases of beans in Africa are anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., angular leaf spot (*Phaeoisariopsis griseola* (Sacc.) Ferraris), rust (*Uromyces appendiculatus* F. Strauss), common bacterial blight (*Xanthomonas phaseoli* (Erw. Smith) Dowson), several common bean viruses transmitted by beetles and white flies, and bean root rots caused by a complex of pathogens (Schwartz and Pastor-Corrales, 1989). It is estimated that 10% of the total yield loss in common bean is caused by bean pathogens (Schwartz *et al*., 2005), and is estimated at 2,288,000 tons annually in Africa (Wortmann *et al*., 1998).

1.3.1 Importance of the bean anthracnose disease

Bean anthracnose is a fungal disease of the common bean caused by *Colletotrichum lindemuthianum* (Sacc. et Magn.) Scrib. The disease is devastating and can cause complete yield losses on susceptible cultivars or when contaminated seed is planted and favorable conditions prevail (Benard-Capelle *et al*., 2006). It is reported to cause greater yield losses in temperate

and subtropical zones than in the tropics (Pastor-Corrales and Tu, 1989). It was once considered the most important disease in bean producing areas of Eastern USA and losses amounting to $1.5 million dollars were reported in Michigan State. The disease has been responsible for significant economic losses in North, Central and South America, Europe, Africa, Australia and Asia (Tu, 1983). Yield losses of up to 95% were recorded in Columbia and over 92% in Malawi (Allen, 1983). In Tanzania yield losses of 40 – 80% were reported and estimated to be worth $304 million dollars per annum (Mohammed, 2013). In Uganda yield losses of up to 30 - 70% were reported (Nkalubo *et al*., 2007). Although not a major problem in developed countries due to effective use of clean seed and resistant varieties, bean anthracnose disease remains a serious and major disease throughout the tropical regions of Latin America and Africa.

1.3.2 Distribution of *C. lindemuthianum*

Bean anthracnose occurs worldwide and is reported wherever the common bean is grown. It was reported in USA (Zaumeyer and Thomas, 1957), European countries (Hubberling, 1977), Canada (Tu and Aylesworth, 1980), Latin America (CIAT, 1988) and in Africa particularly in Uganda, Kenya, Tanzania, Rwanda, Burundi, Ethiopia and D.R Congo. Infected seeds are the commonest means of dissemination of the pathogen causing bean anthracnose disease (del Rio and Bradley, 2004), which in part explains its worldwide distribution.

1.3.3 Anthracnose symptoms and epidemiology

Symptoms of attack by *Colletotrichum lindemuthianum* comprise of dark, sunken, lenticular necrotic lesions containing the acervuli of the pathogen. The initial symptoms of anthracnose appear as a dark brown to black lesion along the veins on the underside of the leaves (Buruchara *et al*., 2010) and susceptible genotypes may exhibit symptoms on all aerial parts of the plant (CAB International, 2004). Seed borne infection usually induces dark brown to black eye-shaped lesions longitudinally on the hypocotyls and cotyledons. On the hypocotyls the lesions enlarge and may cause the stem to break. On older stems, lesions are sunken and may reach 5 – 7mm. Earlier signs of leaf infections occur on the petiole and on the lower leaf surface (Plate 1.1a & c), where small lesions extend along the veins developing a brick red to purple red coloration becoming black. Later similar symptoms appear on the upper leaf surface (CAB International, 2004).

Plate 1.1: Bean anthracnose disease symptoms; **a** = Leaf symptoms; **b** = pod lesions; **c** = stem and petiole symptoms

The most striking symptoms occur on the pods where pod lesions are typically sunken and are encircled by a slightly raised black ring surrounded by a reddish-brown boarder (Plate 1.1b). Under severe infection, young pods may shrivel and dry prematurely (Buruchara *et al*., 2010). The fungus may also penetrate the seed coat and become firmly established within the seed which, when planted serve as the source of infection in the succeeding crop (Buruchara *et al*., 2010).

Bean anthracnose is favored by cool and wet weather with temperatures of 17 – 24^{o}C (Tu and Aylesworth, 1980). Good infection is obtained when inoculated plants are incubated at or near 100% relative humidity at 22^{o}C for 5 – 7 days after artificial inoculation (Pastor-Corrales *et al.*, 1985). The spread of the disease from a focus to other susceptible plants is influenced by environmental factors responsible for inoculum dispersal, such as rain-splash or wind-blown rain and through movement of insects, animals and man, especially when the foliage is moist (Buruchara et al., 2010). The number of foci of the initial inoculum has been shown to be linearly related to disease incidence on leaves but not on pods. Subsequent pod and seed infections can bring on epidemics (Kumar et al., 1999).

1.3.4 Pathogenicity characterization of *C. lindemuthianum*

Identification of *C. lindemuthianum* races is based on reactions of a set of differential cultivars differing in their genes for resistance or susceptibility to one or more races of the pathogen. The occurrence of pathogenic variability in plant pathogens has direct implications on breeding for resistance and therefore the methodologies employed in race determination, (Buruchara, 1991). This is done in order to avoid the possibility of giving the same race different names and vise versa. In order to obtain consistent and comparable results among different workers, the differential cultivars used internationally standardized, genetically pure and able to give clear resistant or susceptible reaction. A set of 12 differential cultivars and a system of nomenclature were proposed and adopted for use in identification of races of *C. lindemuthianum* by a team of bean workers in Latin America during the first Latin American workshop on anthracnose held at Cali, Colombia in 1988 (Buruchara, 1991). The system of nomenclature that was adopted is called the binary system, which identifies a race by a number that is a result of sum of all binary values associated with a cultivar when it gives a susceptible reaction to the race.

The adoption of this standard procedure enabled comparison of data from different research groups worldwide. However, the major limitation was that the experiments depended heavily on environmental conditions and in situations where there is no optimization of experimental conditions; the resulting data might not be satisfactory.

1.3.5 Molecular characterization of *C. lindemuthianum*

The use of molecular techniques for the detection of genetic variability offers a complementary and alternative solution for determining genetic variability in pathogen populations. Molecular tools have been used to study variability of *C. lindemuthianum* at a genetic level including Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Microsatellites (RAMs) and primers derived from the DNA sequence of repetitive-elements (Rep)-PCR which uses primers derived from the 'enterobacterial repetitive intergenic consensus' (ERIC) sequence and the conserved repeated bacterial DNA element 'BOX' for analysis (Mahuku and Riascos, 2004). Using these molecular tools, great genetic variability of *C. lindemuthianum* has been observed and reported. Combining pathogenicity and molecular analysis leads to a better understanding of variability in

C. lindemuthianum. However, a lack of association between pathogenicity data derived from screening from differentials and molecular-derived data has been observed in many cases (Balardin *et* al., 1997; Sicard *et al.*, 1997; Mahuku and Riascos, 2004). This necessitates further investigation to be able to explain the lack of association and further recommend a more reliable set of bean differentials for *C. lindemuthianum* characterization.

1.3.6 Host-pathogen co-evolution

Host-pathogen gene-for-gene (GFG) coevolution between common bean, *Phaseolus vulgaris,* and its pathogen *C. lindemuthianum* was reported to be responsible for the differentiation of host resistance in the centres of diversity of common beans (Gepts, 1988; Geffroy *et al*., 1999; Alzate-Marin *et al*., 1999; and Chiorato *et al*., 2006) and therefore affects the deployment strategy of resistance genes. It was observed that physiological races of *C. lindemuthianum* of Mesoamerican origin were more virulent in both Mesoamerican and Andean bean genotypes while Andean races were virulent only in Andean genotypes (Balardin and Kelly (1998). It was also observed that resistance genes of Mesoamerican origin were highly effective when transferred to beans of Andean background and deployed in regions where Andean isolates prevailed such as East Africa, Colombia, and Ecuador (Miklas *et al*., 2006), while genes of Andean origin were very effective when transferred to beans of Middle American background and deployed in regions where isolates of Mesoamerican origin prevailed such as Central America, Mexico and USA (Miklas *et al*., 2006). There is still a gap in knowledge and understanding of the effectiveness of the individual single resistance genes and effectiveness of pyramided genes in different combinations against the *C. lindemuthianum* population in Uganda.

1.4 Host plant resistance to bean anthracnose disease

The use of host plant resistance is reported as the most effective, safe and inexpensive strategy for the control of bean diseases (CAB International, 2004; Otsyula *et al.*, 2005) through the development and use of cultivars with specific genes. However, this requires continuous identification and characterization of new disease resistance genes and their introduction into commercial bean varieties (Vidigal Filho *et al*., 2007).

1.4.1 Host resistance genes

Resistance to bean anthracnose is conditioned by thirteen (13) major genes *Co-1* to *Co-13* (Lacanallo *et al*., 2010) with only *co-8* being recessive. Loci *Co-9/Co-3*^{3} and *Co-7/Co-3* are allelic (Méndez-Vigo *et al*., 2005) and multiple alleles exist at the *Co-1, Co-3, Co-4, Co-5* loci. Genes *Co-1, Co-12* and *Co-13* are Andean in origin while the rest are Mesoamerican genes (Kelly and Vallejo, 2004) representing the two centers of origin of common beans (Table 1.1).

Table 1.1: Bean anthracnose resistance genes, their sources and linked markers

Gene symbols		pedigree	Gene pool	Linked markers	Map location
Original	**New**				
A	*Co-1*	MDRK	A	$OF10_{530}$	B1
	Co-1^{2}	Kaboon		$SE_{ACT/MCCA}$	
	Co-1^{3}	Perry Marrow		-	
	Co-1^{4}	AND 277		-	
	Co-1^{5}	Widusa		$OA18_{1500}$	
Are	*Co-2*	Cornell 49242	MA	$OQ4_{1440}$, $OH20_{450}$, $B355_{1000}$	B11
Mexique 1	*Co-3*	Mexico 222	MA	-	B4
	Co-3^{2}	Mexico 227	MA	-	
Co-9	*Co-3*^{3}	BAT 93	MA	-	
Mexique 2	*Co-4*	TO	MA	SAS13, SH18	B8
	Co-4^{2}	SEL 1308	MA	SBB14, OC8	
	Co-4^{3}	PI 207262	MA	OY20	
Mexique 3	*Co-5*	TU	MA	$OAB3_{450}$	B7
	Co-5^{2}	SEL 1360	MA	SAB3	
Q	*Co-6*	AB 136	MA	$OAH1_{780}$, $OAK20_{890}$	B7
-	*Co-7*	HI, MSY 7-1, G2333	MA	-	-
-	*co-8*	AB 136	MA	OPAZ20	-
-	*Co-9*	BAT 93	MA	SB12	B4
-	*Co-10*	Ouro Negro	MA	F10	B4
-	*Co-11*	Michelite	MA	-	-
-	*Co-12*	Jalo Vermelho	A	-	-
-	*Co-13*	Jalo Listras Pretas	A	OPV20 700	B3

A = Andean; MA = Meso American; Source: Kelly (2010)

The independent resistance genes *Co-1, Co-2, Co-3, Co-4, Co-5, Co-6, Co-7, co-8, Co-9,* and *Co-10* are present in the cultivars Michigan Dark Red Kidney (MDRK), Cornell 49– 242, Mexico 222, TO, TU, AB 136, G2333, BAT 93, and Ouro Negro, respectively (Alzate-Marin et al., 2003). Alleles *Co-1, Co-1*^{2}*, Co- 1*^{5}*, Co-3*^{2} and *Co-4*^{2} are reported in the cultivars Perry

Marrow and Kaboon, Widusa, Mexico 222, and SEL 1308, respectively (Kelly and Vallejo, 2004).

1.4.2 Marker assisted selection (MAS) in resistance breeding in common beans

DNA markers have enormous potential to improve the efficiency and precision of conventional plant breeding through marker-assisted selection (MAS), which is proven to speed up breeding through laboratory based selection of individual plants with the desired trait(s). MAS has been widely used in development of new varieties that are better adapted to biotic and abiotic constraints. It has been used in common beans to improve germplasm and develop varieties with enhanced resistance against diseases (Miklas *et al*., 2006). Sequence Characterized Amplification Region (SCAR) markers were reported among the most useful of molecular markers in plant breeding programs (Young *et al.,* 1998) and have been used widely in MAS (Garzon *et al.* 2008). The SCARs SAS13, SH18, SBB14, SAB3, SB12 tag the loci/alleles *Co-4*, *Co-4*^{2}, *Co-4*^{2}, *Co-5 and Co-9* respectively for anthracnose resistance. However, there is no reported marker specifically tagged to the *Co-4*^{3} allele which occupies the same locus at the *Co-4*^{2} allele and specific linked markers for the *Co-7* resistance gene in cultivar G2333 are yet to be identified.

1.5 Problem statement

Global yields of common beans are reported to have declined since the early 2000s (FAOSTAT, 2008), with yield of 3 tons/ha under experimental conditions and average of 500 – 700kg/ha under farmer field conditions throughout the tropics. In Uganda a yield difference of approximately 2.0 tons/ha was observed when yields under farmer field conditions and yields realized with improved varieties under experimental conditions were compared (Kalyebara, 2008), indicating declining yields at farm level. Ten percent (10%) of the observed yield decline was attributed to bean diseases (Schwartz *et al*., 2005). Bean anthracnose is the second most important disease of common beans in Uganda causing yield losses of 30 - 70% (Nkalubo *et al*., 2007). Control of the disease using host-plant resistance is recommended as the most cost-effective and environmentally safe strategy. However, *Colletotrichum lindemuthianum,* the causative pathogen, possesses a high degree of genetic and physiologic variability (Sharma *et al*., 1999) leading to major complication in its management due to easy breakdown of single

gene resistance (Melotto *et al*., 2000). Effective control through breeding requires continuous surveillance of *C. lindemuthianum* population structure to understand its current pathogenic variability and distribution. There is need, therefore, to update *C. lindemuthianum* variability in Uganda and identify new sources of effective resistance to the current pathogen structure for use in breeding programs in Uganda.

More than 13 major anthracnose resistance genes were reported from diverse common bean cultivars (Lacanallo *et al*., 2010), but no known single gene was reported to confer resistance to all known races of the *C. lindemuthianum*. Moreover, breakdown of single gene resistance due to emergence of new races is well documented such as in the case of the Andean *Co-1* and Mesoamerican *Co-2* genes, which were reported to breakdown, due to occurrence of new races 31 and 89, after being incorporated into susceptible cultivars (Kruger et al., 1977). Resistance gene pyramiding is, therefore, the most plausible means for achieving broad-spectrum and durable resistance against diverse *C. lindemuthianum* races. The parents G2333 and PI207262 possess natural gene pyramids "*Co-4*^{2}*+Co-5+Co-7*" and "*Co-4*^{3}*+Co-9*" (Kelly and Vallejo, 2004), but their small seed type is not desirable in Uganda. However, their resistance genes could still be exploited. Therefore, there is need for information on the effectiveness of single resistance genes *Co-4*^{2}, *Co-5*, *Co-9* and *Co-4*^{3} and pyramided gene combinations "*Co-4*^{2}*+Co-5+Co-9*", "*Co-4*^{3}*+Co-5+Co-9*", "*Co-4*^{2}*+Co-9*" and "*Co-5+Co-9*" against *C. lindemuthianum* in Uganda.

Molecular markers are used to facilitate and speed up the process of gene pyramiding. However, most of the available markers for anthracnose resistance genes have not been validated in Uganda. There is also a challenge of tracking the *Co-4*^{3} allele since it lacks a specific marker that tags it especially where both *Co-4*^{2} and *Co-4*^{3} alleles are segregating. There is also no linked marker reported for tracking *Co-7* gene in the cultivar G2333 making it difficult to track during marker assisted selection. Disease resistance is reported to cost the plant in terms of yield (McGrann *et al*., 2014), and therefore, there is need elucidate on whether pyramiding anthracnose resistance genes in common bean genotypes has a negative effect on yield traits.

1.6 Justification

Understanding the current *C. lindemuthianum* variability in Uganda would reveal the existing pathological races and their distribution and the information would be important to breeders and pathologists. The use of standard differential cultivars to characterize *C. lindemuthianum* pathotypes would also reveal new sources of effective broad-spectrum resistance suitable for use in breeding programs in Uganda.

The use of molecular techniques would be more practical in gene pyramiding as the identification of plants carrying two or more resistance alleles of different genes would be made easy thus easing the constraints of classical breeding which is limited by the length of screening procedures and reliance on the environmental factors.

Pyramiding resistance genes to bean anthracnose disease and assessing the different single genes and pyramid gene combinations would reveal information on the most effective single genes and the most effective pyramid gene combinations with broad-spectrum resistance against *C. lindemuthianum* in Uganda. The populations/ lines developed would be taken up by the National bean breeding program for use as elite breeding materials, while others would be evaluated for possible release as commercial varieties which would offer Ugandan farmers reduced yield losses as a result of effective resistance against bean anthracnose disease.

Understanding the relationship between number of pyramided resistance genes and yield would give insight in to whether the gene pyramiding may have a cost yield through negatively affecting yield traits. This information would guide breeders in making designing gene pyramiding strategies that would maximize yield gain among progenies and limit the potential yield cost.

1.7 Overall objective

The overall objective of the study was to contribute to the understanding of gene pyramiding for broad-spectrum resistance to bean anthracnose disease in Uganda.

1.8 Specific objectives

1. To determine the pathogenic variability of *Colletotrichum lindemuthianum* in Uganda
2. To evaluate effectiveness of pyramided resistance genes against bean anthracnose disease

3. To determine the genetic variability and relationship between number of pyramided resistance genes on yield traits among advanced common bean populations

1.9 Hypotheses

1. *Colletotrichum lindemuthianum* has a high pathogenic variability in Uganda and is location specific
2. Resistance gene pyramiding for bean anthracnose is effective in conferring broad-spectrum resistance to bean anthracnose disease
3. A high genetic variability exists among advanced common bean populations and number of pyramided genes has no negative relationship with yield traits

CHAPTER TWO

Literature review

2.1 Variation of *Colletotrichum lindemuthianum*

There are many anthracnose pathotypes or physiological races identified by their pathogenicity reactions on a set of host differentials. The highest number of *C. lindemuthianum* races was reported in the two centers of diversity of common beans namely the Andean and Mesoamerican regions (Gepts, 1988; Pastor-Corrales *et al*., 1995).

Barrus made the first report of α (alpha) and β (beta) races; Burkholder described the γ (gamma) race, while Andrus and Wade reported the δ (delta) race; Hubbeling reported the δ race, Schnock indicated κ (kappa) race (Leaky and Simbwa-Bunya, 1972) and Tu (1992) proposed existence of clearly different physiological races of *C. lindemuthianum*, including α, β, δ, ε, γ, κ and λ (lambda). Kelly et al. (1994) reported two races, 7 and 73, in Michigan and North Dakota. Since race 73 overcomes the *Are* gene and race 7 overcomes the *A* gene, both of which were extensively used in the breeding program the use of gene pyramiding as a disease resistance strategy was suggested, since the *A*/*Are* gene combination afforded resistance to both races.

Using the binary nomenclature system, 38 races were reported in Mexico (Rodriguez, 1991), seven races were identified in a group of 10 isolates from Nicaragua (Rava *et al*., 1993), 33 were characterized from a group of 178 isolates from Colombia (Pastor-Corrales *et al*., 1995) and three races were described in the United States (Balardin and Kelly, 1996). Sicard et al. (1997a) studied population subdivision of *C. lindemuthianum* in the Mexican, Ecuadorian and Argentinean bean populations representing the centers of diversity of common beans. They used Random Amplified Polymorphic DNA (RAPD) markers and virulence on 12 differential cultivars to assess diversity of the pathogen. Findings revealed significant differentiation of the three regions with Mexico having the highest polymorphism. Fifteen physiological races were identified out of 45 isolates collected from five wild common bean populations in the South Andean center of origin.

Balardin et al. (1997) used the 12 differential bean cultivars to characterize 138 *Colletotrichum lindemuthianum* isolates collected from Argentina, Brazil, the Dominican Republic, Honduras,

Mexico, and the United States and forty one (41) races were identified and categorized into two groups: those found over a wide geographic area and those restricted to a single country. Races 7, 65, and 73 were widespread with race 73 being the most common while race 7 was found once in Argentina and Mexico but at a higher frequency in the United States. Race 65 was found repeatedly in Brazil and the United States. Although 39% of the races were detected repeatedly and three races were widespread, no race was isolated from both *P. vulgaris* gene pools. Phenetic analyses showed no obvious patterns correlated with virulence clusters and no geographic pattern was evident. Molecular polymorphism generated by RAPD markers confirmed the extensive variability in virulence of *C. lindemuthianum*. Virulence phenotypes were grouped into 15 clusters with the two largest clusters containing isolates from all the geographic regions sampled. Molecular polymorphism was observed among isolates from races 65 and 73 within and among countries, except among Brazilian isolates of race 65. The genetic diversity of *C. lindemuthianum* was greatest in Mexico and Honduras.

Sharma et al. (1999) collected 85 isolates of *C. lindemuthianum* from Himachal Pradesh state of India and characterized them based on their reaction on the standard differential cultivars and CIAT differentials. Using the standard differential cultivars, 12 races were characterized and the races designated IndI and IndIX were different from those identified in Europe and USA forming a new race group from the Indian Subcontinent. Using the CIAT differential set 19 races were identified and only races 65 and 73 were similar to the races reported in North American. Padder et al. (2007) analyzed five *C. lindemuthianum* populations in Himachal Pradesh state, India for genetic diversity on the basis of allele frequencies of 12 RAPD markers using Nei's genetic diversity formulae. They reported diversity within each population to be high with values ranging from 0.26 – 0.31.

Mahuku and Riascos (2004) assessed genetic variability of 200 *Colletotrichum lindemuthianum* isolates collected from the Andean and Mesoamerican bean varieties and regions. They observed high levels of *C. lindemuthianum* variability and genetic diversity (0.97) with 90 races identified from the 200 isolates, revealing that *C. lindemuthianum* is a highly diverse pathogen. Ansari et al. (2004) characterized pathogenic and genetic diversity of *Colletotrichum lindemuthianum* isolates collected from a total of 10 Central and South American, European and African countries. They used pathogenicity tests on the standard differential cultivars and

Amplified Fragment Length Polymorphism (AFLP) analysis. On the basis of pathogenicity tests, 74 isolates were attributed to 30 different pathogenic races using the CIAT-defined binary race-classification system. Race 9 was the most widespread, being detected in four different countries. Cluster analysis of consensus AFLP data generated using three selective AFLP primer combinations grouped 86 isolates including the 74 subjected to pathogenicity tests into three clusters. This analysis showed that the majority of South and Central American isolates were divided among two clusters, and that limited number of European and African isolates used in this study were genetically most similar to Central American isolates.

Alzate-Marin et al. (2004) reported a total of 50 *C. lindemuthianum* races identified in Brazil between the period 1996 and 2002 by use of standard differential bean cultivars. Races 65, 73, 81 and 87 were the most frequent and widely distributed in the country, and were commonly found in the States of Paraná, Santa Catarina, Góias and Distrito Federal. Pathotypes 1, 5, 17, 67, 79, 85, 86, 93, 96, 102, 105, 111, 117, 121, 123, 125, 137, 193, 217, 320, 321, 339, 343 and 585 were identified in only one Brazilian State. Although the number of collected isolates was different from each State, data showed that the Paraná State presented the highest *C. lindemuthianum* variability with 29 races followed by the States of Goiás with 17 races, Santa Catarina with 16 races and Rio Grande do Sul with 14 races.

Talamini et al. (2006) assessed genetic divergence of *C. lindemuthianum* using RAPD markers. Thirty one (31) isolates were collected from three regions of Minas Gerais state in Brazil. Three isolates of the sexual phase of *C. lindemuthianum* (*Glomerella cingulate* f.sp *phaseoli*) were included in the study. Band patterns generated using 11 primers produced 133 polymorphic bands, which were used to determine genetic divergence among and within the pathogen races. The isolates analyzed were divided into six groups with 0.75 relative similarity. Group IV formed by the three isolates of the sexual phase was the most divergent and races previously determined using differential cultivars did not correlate with the results obtained using RAPD markers.

Munda et al. (2009) used AFLP markers to assess genetic variability and relationships among *C. lindemuthianum* isolates from Slovenia that had been characterized as races 23, 55, 103 and 131. AFLP clustering revealed two main groups that did not clearly correspond to race

classification based on pathogenicity towards standard differential cultivars. Ribeiro et al. (2016) collected 51 isolates from the states of Sao Paulo and Santa Catarina, Brazil and tested for physiological races and for variability among isolates of the race 65 using the 12 standard differential cultivars. They identified 10 physiological races 4, 38, 55, 65, 73, 81, 83, 85, 321, and 351. Races 65 and 81 predominated, with frequencies of 37.25 and 35.29%, respectively. Regarding the isolates of race 65, wide physiological variability was evident. Greater occurrences of races 65, 73, and 81 were observed. Mota et al. (2016) studied the variability of *C. lindemuthianum* and its Telemorph *Glomerrela spp* in common beans using morphological traits such as index of mycelial growth, colony diameter and sporulation capacity; pathogenicity tests and Interretroelement Amplified Polymorphism (IRAP) markers. They observed a high level of variability of *C. lindemuthianum.*

Bigirimana et al. (2000) used the 12 standard differentials to characterize 12 isolates collected from bean growing areas in Burundi. Detached unifoliated bean leaves from 8-day old plants were inoculated and incubated under controlled conditions for seven days. Symptoms were scored on a 1-9 scale. A high diversity of *C. lindemuthianum* was observed with nine (9) races namely 9, 69, 87, 384, 385, 401, 448, 449 and 485 identified. Seven of these races (9, 69, 87, 384, 401, 448, and 485) were reported for the first time in Africa and races 401 and 485 were reported for the first time in literature.

In Uganda, Leaky and Simbwa-Bunnya (1972) using differential cultivars from Shreiber and Hubberling, identified races 17, 19, 23, 102, 130, and 453 with isolates collected from Central, Western and South Western regions of Uganda. More recently, Nkalubo (2006), using the standard set of 12 differential cultivars, reported eight races 23, 55,102, 130, 227, 375, 511 and 767 from Kabale, Kisoro, Bushenyi and Mpigi districts with race 767 reported as the most widespread and virulent. Mwesigwa (2008) used twenty RAPD markers, two rep-PCR primers and virulence on a set of 12 standard differential common bean cultivars to assess the genetic and physiological diversity of 74 *C. lindemuthianum* isolates from Kabale, Mbale, Apac, Mpigi and Wakiso districts. Analysis of molecular data resulted into 3 major clusters. Comparison of results from the race differentials with those from the molecular techniques did not show a strong relationship. Pathotype characterization of 47 isolates with differential cultivars revealed 21 races namely 0, 2, 3, 4, 6, 14, 128, 262, 264, 268, 320, 452, 481, 1024, 1536, 1538, 1856,

1857, 1989, 3086 and 4033. None were similar to those reported by Leaky and Simbwa-Bunnya (1972) and Nkalubo (2006). Nine of them infected Mesoamerican cultivars, three infected Andean cultivars and seven infected cultivars from both gene pools while race 0 did not infect any of the differential cultivars. Races 1024, 1536, 1538, 1856, 1857, 1989, 3086 and 4033 were the most virulent as they incited symptoms on either one or both of the highly resistant differential cultivars AB136 and G2333 with an average severity level of 4. Races 0 and 6 were the most wide spread; they were isolated from the districts of Kabale, Mbale and Apac (race 0) and Mpigi and Kabale (race 6).

2.2 Resistance gene pyramiding and its effectiveness

REX Consortium (2016) investigated the deployment strategy that results in the greatest durability of resistance genes. They reported that although theoretical and empirical studies comparing deployment strategies of more than one resistance gene are very scarce, overall durability of disease resistance genes can be increased by combining their presence in the same plant through pyramiding. Their retrospective analyses of field monitoring data suggested that the pyramiding of disease resistance genes within a plant was the most durable strategy and by extension, suggested the combination of disease resistance genes with other practices for pathogen control such as use of pesticides and best cultural practices as a relevant management strategy to slow down the evolution of virulent pathotypes.

Caporalino et al. (2014) evaluated three strategies to control root-knot nematodes in lettuce using cultivar mixtures, alternating resistance genes and pyramiding resistance genes, under controlled and field conditions. Results showed that the choice of the single resistance gene and the genetic background in which it was introgressed affected the frequency of resistance breakdown and that pyramiding of two resistance genes in one genotype suppressed the emergence of virulent isolates. Results further revealed that alternating different resistance genes in rotation was efficient in decreasing virulent populations of nematodes in fields due to the specificity of the virulence and the trapping effect of resistant plants; however, mixing resistant cultivars appeared as a less efficient strategy to control nematodes. These findings help demonstrate the general applicability of such strategies for breeding and sustainable management of resistant cultivars against pathogens.

Kelly et al. (1994) suggested that pyramiding the *Co-1* Andean and the *Co-2* Mesoamerican genes would afford protection against all known *C. lindemuthianum* races in North America. Young and Kelly (1996) suggested that deployment of major genes such as *Co-6* and *Co-5* in different combinations with *Co-1* gene could contribute to more durable anthracnose resistance in common beans.

Kelly et al. (1995) reported on the use of gene pyramiding in common beans for the development of more effective resistance to the temperature-insensitive-necrosis-inducing (TINI) strains of Bean Common Mosaic Virus (BCMV) present in the USA. Contrary to previous work, they indicated that the *bc-3* gene was effective against these strains in the absence of the strain unspecific *bc-u* gene in genotypes possessing the *I* gene. The epistatic *bc-3* gene was observed to interfere with traditional efforts to pyramid the other recessive resistance genes by masking their activity. They recognized the fact that indirect selection based on markers linked to the other recessive resistance genes would be ineffective without the ability to select for the *bc-u* gene which is required for expression of the *bc-22* gene in germplasm carrying the *I* gene and that the most resistant genotype (*I, bc-u, bc-I 2, bc-22, bc-3*) can only be introduced into a wide range of germplasm through the use of molecular markers linked to the different resistance genes.

Scully et al. (1995) conducted a breeding program in the USA with an objective to pyramid a series of resistance genes to multiple viruses into a genetically diverse set of common bean breeding lines with the assumption that by developing an elite set of breeding lines with broad-spectrum virus resistance, a gene or group of genes can be more easily incorporated into existing regional cultivars. The specific viruses addressed by this effort included bean common mosaic (BCMV), bean yellow mosaic (BYMV), broad bean wilt (BBWV), blackeye cowpea mosaic (BlCMV), clover yellow vein (CYVV), pea mosaic (PeMV), soybean mosaic (SMV), tobacco mosaic (TMV), and watermelon mosaic (WMV) viruses. They made a cross between Great Northern-1140 (GN-1140) and B-21 and used a modified single-seed descent scheme to successfully develop and officially release five common bean lines [SP 6C, SP 17B, SP 61, SP 180D and SP 377C] resistant to all the above nine viral diseases.

Kelly (2004) further reviewed highlights of the work that led to advances in common bean improvement in the U.S. and other countries and particularly the classical and molecular pyramiding of major genes for resistance to anthracnose, bean common mosaic virus, rust, and QTL for resistance to common bacterial blight to provide for enhanced durability of resistance. He further identified one of the major challenges in bean improvement for the future, especially in the developing world, to be the feasibility of incorporating new biotechnology tools such as molecular markers and MAS as the technologies demand increased costs and facilities associated with an increased level of uncertainty regarding outcome and usefulness.

Liebenberg et al. (2005) reported the successful transfer of common bean rust resistance genes from 16 donor lines to adapted germplasm under the dry bean breeding program of the Agricultural Research Council in South Africa since the1970s. The pyramided resistance genes include *Ur-3+, Ur-5, Ur-11* and other uncharacterized genes. However, to protect the durability of the resistance sources used, emphasis was placed on concurrent research to combine several genes in a single well-adapted background.

Asensio et al. (2005) investigated broadening the genetic base and breeding for higher yielding multiple disease resistant Andean cultivars using inter-gene pool populations by studying the feasibility of introgression of resistance to *bean common mosaic virus* (BCMV), common bacterial blight (*Xanthomonas campestris*) and halo blight (*Pseudomonas syringae*) using gamete selection. They also investigated the relative importance of the use of landrace cultivars versus elite breeding lines as the last parent in making maximum genetic contribution in multiple-parent inter-gene pool crosses for breeding for resistance to diseases. Single plant selections were made from F1 to F5 generations for resistance to BCMV, common bacterial blight and halo blight. They evaluated parents and $F_{5:6}$ lines for BCMV, common bacterial blight, halo blight, growth habit and 100-seed weight. Seven F_6 lines with both common and halo blight resistance and only one F_6 line resistant to all the three diseases were developed. It was observed that none of the selected breeding lines had seed sizes as large as the largest Andean parent and that an elite breeding line rather than a landrace cultivar was the most suitable as the last parent in the crossing scheme making maximum genetic contribution to the multiple-parent inter-gene pool crosses and simultaneous selection for plant type, seed traits as well as resistance to diseases.

Pastor-Corrales et al. (2007) reported six great northern bean germplasm lines pyramided with rust and mosaic resistance genes, including lines BMN-RMR-8, BMN-RMR-9, BMN-RMR-10, BMNRMR-11, BMN-RMR-12 and BMN-RMR-13, which were developed by the United States Department for Agriculture (USDA) – ARS Beltsville Agricultural Research Centre. These were reported as the only great northern bean lines in the world to combine in pyramid four genes *Ur-3, Ur-4, Ur-6,* and *Ur-11* known to confer resistance to all known races of the highly variable bean rust pathogen with the *bc-3* resistance gene that conditions an immunity to all strains of the seed-borne bean common mosaic (BCMV) and bean common mosaic necrosis (BCMNV) viruses.

In order to combat white mold of common beans in the USA, Tera'n and Singh (2010) successfully introgressed high levels of white mold resistance from the secondary gene pool through backcrossing. They pyramided these resistance genes and developed common bean cultivars with high levels of white mold resistance combined with common bacterial blight (CBB), bean common mosaic virus (BCMV) and bean curly top virus (BCTV) resistances. Allele selection was achieved through phenotypic screening involving use of multiple inoculations with multiple isolates of *Sclerotinia sclerotiorum*, filed evaluations and verification of the resistance response at harvest.

Ragagnin et al. (2009) used RAPDs and SCAR markers to pyramid *Co-4, Co-6, Co-10* anthracnose, *Phg-1* angular leaf spot and *Ur-ON* rust resistance genes in to a susceptible 'carioca' market class cultivar in Brazil. They used TO, AB 136, AND 277 and Ouro Negro as donor parents in a backcross scheme and molecular fingerprinting to select the lines genetically closer to the recurrent parent. Intercrossed BC lines showed that four $F_{4:7}$ pyramid lines had inherited all the target resistance from their respective donor parents against the respective pathogens of the three diseases.

Genchev et al. (2010) combined various genes for resistance to anthracnose in Bulgaria using alternating crossing which resulted in recombinant inbred lines (RILs) based on the cross DG 2-36 [Red Hawk/TO//AB136/3/Red Hawk/TO//TU/4/DG 98-53]. Several races of *C. lindemuthianum* identified in Bulgaria were used for phenotypic screening from F_1 to F_5

generation. Seven RILs of F_6 progeny, possessing more than one specific gene for resistance were selected by RAPD and SCAR markers linked to *Co-1* and *Co-4* genes. The line DG 2-36-58-3 was identified as the most promising in growth habit type, maturity period, seed type, yield and resistance to anthracnose provided by pyramided *Co-1* and *Co-4* genes.

Sofkova et al. (2010) reported a half-century genetic and breeding program that aimed at improving common beans in Bulgaria while addressing the key diseases namely bacteriosis, white mold, anthracnose, rust, and viruses like BCMV, CMV, BYMV, ClYVV. Biotechnological approaches and in vitro techniques were successfully used to pyramid major resistance genes obtained from *P. coccineus* and *P.acutifolius* mutant lines into susceptible cultivars and subsequently several cultivars were successfully developed as a result of combining selection for multiple disease resistance. Three of these cultivars were recommended for release and commercialization in the Bulgarian vegetable market for fresh consumption, processing and shipping.

Rocha et al. (2012) assessed SCAR markers and found them to be efficient in selecting plants resistant to both anthracnose and angular leaf spot. Twenty six superior families possessing the highest number of markers were identified using SCAR markers and selected in the F_4 generation. Eighteen of these families were resistant to the races 65 and 453 *of Colletotrichum lindemuthianum* and five were resistant to the race 63.23 of *Pseudocercospora griseola.*

Ferreira et al. (2012) used SCAR, CAPs and RAPD markers to successfully pyramid *Co-2, Co-3/9* anthracnose, *I* and *bc-3* common mosaic virus resistance genes into a market class bean genotype.

Singh et al. (2014) successfully pyramided high levels of white mold resistance into pinto beans in the USA using multiple-parent populations derived from four sets of crosses [SE154 (VA 19/MO 162//A 195/G 122); SE152 (CORN501/G122//A 195/VCW 55); SE153 (USPT-WM-1/CORN 601//USPT-CBB-1/ 92BG-7); and SE155 (A195/4/NY6020-4/92BG-7///MO 162/I9365-25//ICA Bunsi/G 122] between and within gene pools, involving seven Andean and six Mesoamerican parents. Four large-seeded, < 40g/100 seeds, Andean (SE152-6, SE154-1,

SE154-9, and SE155-9) and four medium-seeded, 25-40 g/100 seeds, Mesoamerican (SE153-1, SE153-3, SE153-6, and SE153-7) pyramided lines with white mould resistance were successfully developed. Phenotypic screening under controlled conditions of the pyramided breeding lines and seven resistant parents using four pathogen isolates ARS12D, ND710, CO467 and NY133 revealed that the percentage of resistant plants for the pyramided breeding lines ranged from 63 to 82% and mean disease severity ranged from 4.0 to 4.6 in contrast to the respective values for the resistant parents of 3 to 57% percentage of resistant plants and 4.8 to 7.5 disease severity.

Wahome et al. (2011) evaluated seven groups of snap bean populations of different generations, and 45 bush snap bean lines for resistance to angular leaf spot, anthracnose, and rust diseases in Kenya. Field evaluation showed that two bush lines (KSB 10 W and KSB 10 BR), and one climbing line (HAV 130) had consistent multiple resistance to angular leaf spot, anthracnose and rust diseases. Nine lines and 674 single plants were selected from populations showing multiple disease resistance. Resistance in selected lines reduced angular leaf spot, anthracnose and rust severity by 17, 16 and 36%, respectively.

Oballa et al. (2012) studied the effectiveness of pyramided resistance genes in improving resistance to Fusarium root rot in susceptible common bean cultivars in Uganda. Crosses were made among the resistant donor lines involving six inbred lines, MLB-48-89A (M48), MLB-49-89A (M49), G2333 (G2) and G685 (G6) and two susceptible cultivars K20 and Kanyebwa. Five-parent and single cross populations were developed and subjected to *Fusarium solani* f. sp. *phasoeli* isolate-3 under controlled conditions. Results indicated that two to three genes were segregating in the populations and that the F_1 and F_2 means of the five-parent cross had lower disease scores though not significantly than the single-crosses from the respective donor parent. F_2 frequency distributions showed that the FPC in both Kanyebwa and K20 populations had higher proportions of resistant plants than any of the single crosses in the respective populations. Their findings revealed that the pyramiding of resistance genes from different Fusarium root rot resistance sources provided a stable source of resistance than using single sources of resistance.

Kivuru, (2013) successfully introgressed anthracnose resistance genes *Co-4*^{2} and *Co-6* into common bean lines with resistance to angular leafspot, common bacterial blight, bean common mosaic virus and bean common necrotic virus. Donor parents C4-1308B-3E-8-B and AB136 were used for *Co-4*^{2} and *Co-6* resistance genes respectively. Highly effective resistance to anthracnose disease was observed among the segregating populations.

Njuguna et al. (2014) successfully used SCAR markers to pyramid multiple resistance genes to angular leaf spot, anthracnose, root rots and bean common mosaic virus into susceptible commercial cultivars in Kenya. Sixteen populations were generated from crosses among six sources of resistance (MEX 54, G10909, G2333, RWR 719, AND 1062 and BRB 191) and four susceptible popular varieties (KAT B1, KAT B9, GLP 585 and GLP 92). Male gametes with requisite resistance genes were identified using markers SAB3 for anthracnose, SH13 for angular leaf spot, *SW13* for bean common mosaic virus, and *PYAA19*$_{800}$ for Pythium root rot, and used to construct the F_1 with susceptible varieties following gamete selection breeding method. Results on marker validation showed that three markers SAB3, SH13 and SW13 were effective selection tools as they amplified the genes and showed polymorphism among the plants, while the marker *PYAA19*$_{800}$ for Pythium root rot did not amplify during PCR reaction and was therefore ineffective in selecting for the gene conferring resistance to Pythium root rot. The three markers were used to screen progenies of the cross G10909/G2333//AND 1062/BRB 191 for presence of resistance genes to the three diseases and out of 89 male gamete plants that were screened only five plants were positive for three markers and 18 for two markers.

Ddamulira et al. (2015) conducted a study to determine the effectiveness of pyramided genes in improving angular leaf spot resistance in susceptible common bean cultivars in Uganda by making crosses among five inbred lines, three resistant donor parents AND277, Mexico 54, G5686 and two susceptible cultivars K132 and Kanyebwa in a cascading gene pyramiding scheme to develop triple crosses (TC). The TC F_1 and each of the resistant parents were crossed with each of the two susceptible cultivars to generate four parent crosses (FPC) and single crosses (SC), respectively. All populations were inoculated with race 61:63 of *P. griseola* under controlled conditions. Results revealed that two to three resistance genes were segregating in the populations and that the resistance to race 61:63 was complex with epistatic effects. The four

pyramided genes in the FPC conferred a more effective resistance against isolate 61:63 than two or three genes.

Mukankusi et al. (2016) reported ongoing efforts under CIAT-Uganda in collaboration with the National Beans Research Program, NaCRRI, to develop multiple disease resistant varieties of common beans by first developing multiple disease resistance parents through pyramiding of key disease resistance genes in a common background. They used SCAR and SSR molecular markers linked to resistance genes to anthracnose, Pythium root rot, angular leaf spot and Bean Common Mosaic Virus (BCMV) diseases in beans. The target genes and sources of resistance used included the *I* and *bc-3* genes for Bean Common Mosaic Virus (BCMV) and its necrotic strain Bean Common Mosaic Necrotic Virus (BCMNV) from genotype MCM5001; *Co-4*^{2}, *Co-5* and *Co-7* genes for anthracnose resistance from genotype G2333; *Prr* gene for Pythium root rot resistance from genotype MLB-49-89A, and the *phg* gene for angular leaf spot resistance from genotype MEX54. They conducted Single and double crosses between these parents and screened up to 1,500 F_2 plants of the single cross and over 3,000 F_2 plants of the double cross using molecular markers so as to select individuals of the root genotype with a seven-gene pyramid of *I+bc-3+Co-4*^{2}*+Co-5+Co-7+Prr+ phg*. Preliminary findings indicated that lines with pyramided genes were more effective as parents in transferring combined resistance to susceptible cultivars than the individual sources.

2.3 Assessment for yield performance and possible yield penalty among pyramided populations

Yield is the single most important indicator of crop performance but disease resistance is reported to be costly on the plant and therefore, has commercial significance because it may hinder the more important objective of increasing yield (Brown, 2002).

Coyne et al. (2000) reported a common bean cultivar ‘Weihing’ in the USA which was bred with multiple resistances to rust, common bacterial blight and white mold using pedigree selection. Field evaluation results revealed no significant differences between the mean yield of the pyramided cultivar and two other standard cultivar checks.

Liebenberg et al. (2005) reported the successful gene pyramiding of three characterized rust resistance genes namely *Ur-3+, Ur-5, Ur-11* and other uncharacterized genes in to advanced common bean lines. Field testing for agronomic performance of the lines showed that resistance was considerably improved without yield loss.

Fininsa and Tefera (2007) evaluated eight (8) genotypes [EMP 219, TY 3396-6, TY 3396-7, TY 3396-12, RAB 404, ARA 21, TAR 3, BZ 1289-12] with multiple resistance for anthracnose, angular leaf spot and common bacterial blight and two commercial checks (Ayenew and Roba-1) in field performance trials. It was revealed that genotypes with multiple resistance were late maturing and the commercial check Ayenew had the highest 100-seed weight (46 g), but the multiple resistant genotype TY 3396-12 had better yield (4.5 t ha^{-1}) implying no yield penalty.

Ragagnin et al. (2009) bred for multiple resistance to anthracnose, angular leaf spot and rust in common beans using marker assisted gene pyramiding techniques. Results revealed that pyramided lines with all the target resistance genes were as productive as the best 'carioca-type' cultivars without gene pyramids.

Souza et al. (2009) used backcross method and marker assisted gene pyramiding to develop advanced common bean lines with wide resistance spectra to rust disease and no yield penalty was revealed in relation to the recurrent parent after evaluating yield components such as grain yield (kg/ha), plant height, seeds per pod and pods per plant. Wahome et al. (2011) evaluated snap bean populations of different generations, for resistance to angular leaf spot, anthracnose, and rust diseases in Kenya and observed some of the advanced lines selected for multiple disease resistance could not meet the yield and quality of bush commercial varieties.

Souza et al. (2014) used DNA markers to develop carioca seeded common bean elite $F_{4:7}$ lines pyramided with three different rust resistance genes *Ur-5, Ur-11 and Ur-14*. Results showed that the selected lines harbouring the three-gene pyramid were as productive as the recurrent parent and high performing control cultivars grown in Brazil.

Mulanya et al. (2014) bred for multiple resistance to rust, anthracnose and angular leaf spot in snap beans and reported four lines with multiple resistance to the three diseases that had better pod yield and pod quality compared with existing commercial varieties in Kenya. Mukankusi et al. (2012, 2016), in their effort to develop multiple disease resistance breeding parents with pyramided resistance genes to anthracnose, Pythium root rot, angular leaf spot and Bean Common Mosaic Virus (BCMV) diseases, observed negative correlations between the number of pyramided genes and several agronomic traits implying a possible yield penalty. Miklas et al. (2017) examined effects of two quantitative trait loci (QTLs) from tepary bean on agronomic and canning quality traits. Plots were inoculated with a mixture of *Xanthomonas axonopodis* pv. *phaseoli* and *X. fuscans* sbsp. *fuscans* isolates. Results revealed that the QTLs *BC420* and *SU91* did not cause a yield penalty.

CHAPTER THREE

Pathogenic variation of *Colletotrichum lindemuthianum* in Uganda

Abstract
Colletotrichum lindemuthianum is a highly variable pathogen of common beans that easily overcomes resistance in cultivars bred with single-gene resistance. Samples of common bean tissues with anthracnose symptoms were collected in eight districts of Uganda, namely Kabarole, Sironko, Mbale, Oyam, Lira, Kapchorwa, Maracha and Kisoro and 51 isolates which sporulated successfully on Potato dextrose Agar and Mathur's media were used to inoculate 12 differential cultivars under controlled conditions. Five plants per cultivar were inoculated with each isolate and evaluated for their reaction using the 1 – 5 severity scale[1] (Inglis *et al*., 1988). Races were classified using the binary nomenclature system proposed by Pastor Corrales (1991). Variation due to cultivar and isolate effects was significant ($P \leq 0.001$) for severity. The 51 isolates from eight districts grouped into 27 different races. Sironko district had the highest number of races followed by Mbale and Kabarole. Races 2047 and 4095 were the most frequently found, each with 10 isolates grouped under them. Race 4095 was the most virulent and caused a susceptible (S) reaction on all 12 differential cultivars and the susceptible check followed by races 2479, 2047 and 2045 respectively. Two races, 4094 and 2479, caused a susceptible reaction on the differential cultivar G2333, which nevertheless, showed the most broad spectrum resistance followed by cultivars Cornell 49-242, TU, and AB136 respectively which were recommended for use in breeding programs aimed at breeding for broad spectrum resistance to bean anthracnose in Uganda.

Key words: Broad spectrum, races, virulence, diversity, pathotypes, differential cultivars

3.1 Introduction

The pathogen *Colletotrichum lindemuthianum* has a wide variation with various races reported in major bean producing regions of Meso-America and Latin America (Balardin and Kelly, 1998) that are the center of origin of common beans (Pastor-Corrales *et al*., 1995). The highest diversity and variation of *C. lindemuthianum* was reported in Latin America (Pastor-Corrales *et al*., 1995). The East African highland region is regarded as the secondary center of diversity of common beans (Schwartz and Pastor-Corrales, 1989) and is expected to have a high variability of *C. lindemuthianum* due to host-pathogen co-evolution. Mahuku and Riascos (2004) assessed virulence and molecular diversity of 200 *C. lindemuthianum* isolates collected from Andean

and Mesoamerican bean cultivars and they reported high levels of pathotypic diversity with up to 90 pathotypes identified. Bigirimana et al. (2000) identified nine *C. lindemuthianum* races namely 9, 69, 87, 384, 385, 401, 448, 449 and 485 from 12 isolates collected from major bean growing areas in Burundi using 12 standard differential cultivars.

In Uganda, three key studies on characterization and diversity of *C. lindemuthianum* were conducted by Leaky and Simbwa-Bunnya (1972), Nkalubo (2006) and Mwesigwa (2008). Using differential cultivars from Shreiber and Hubberling, Leaky and Simbwa-Bunnya (1972) identified races 17, 19, 23, 102, 130, and 453 with isolates collected from Central, Western and South Western regions of Uganda. Nkalubo, (2006) reported races 23, 55,102, 130, 227, 375, 511 and 767 as the most abundant from Kabale, Kisoro, Bushenyi and Mpigi districts. Mwesigwa (2008) reported 21 races (0, 2, 3, 4, 6, 14, 128, 262, 264, 268, 320, 452, 481, 1024, 1536, 1538, 1856, 1857, 1989, 3086 and 4033 from Kabale, Apac, Mbale, Mpigi and Wakiso districts. However, nine (9) years have passed since the last study was conducted and change in diversity of the pathogen could have taken place due to increase in bean production, introduction of new germplasm from different gene pools and movement of bean seed within the country and across borders. This, therefore, necessitated another study so as to understand the current variability of the pathogen. The objective of this study therefore was to determine the current status of the pathogenic variation of *C. lindemuthianum* in Uganda.

3.2 Materials and methods

3.2.1 Collection of *C. lindemuthianum* samples

Different *C. lindemuthianum* isolates were collected from different locations (Table 3.1) in the different parts of Uganda from farmers' fields which were selected purposively depending on presence of bean anthracnose disease. Bean pods with symptoms of anthracnose disease were collected from different cultivars from eight districts of Uganda. A sampling quadrant measuring $1M^2$ was used in the farmers' fields to select plants from a given part of the field, which were used for sample collection. Data was collected on disease incidence and severity.

Table 3.1: *C. lindemuthianum* isolates collected in different parts of Uganda.

District	Sub-county	Village	Longitude	Latitude	Altitude	Isolate I.D*
Kabarole	Mugusu	Kibede	00°36.152N	030°12.976E	1596	025A
Kabarole	Mugusu	Kirugu	00°37.461N	030°13.135E	1605	028A
Kabarole	Mugusu	Kisaru	00°37.506N	030°12.528E	1562	34A
Kabarole	Karambi	Karambi	00°38.107N	030°13.785E	1563	36A
Kabarole	Karambi	Karambi	00°38.107N	030°13.785E	1563	37A
Kabarole	Karambi	Karambi	00°38.107N	030°13.785E	1563	38A
Kabarole	Karambi	Karambi	00°38.107N	030°13.785E	1563	40A
Kabarole	Karambi	Karambi	00°38.100N	030°14.842E	1553	41A
Kabarole	Karambi	Karambi	00°38.100N	030°14.842E	1553	44A
Kabarole	Karambi	Karambi	00°37.923N	030°14.643E	1553	46A
Kabarole	Karambi	Karambi	00°37.923N	030°14.643E	1555	52A
Kabarole	Karambi	Karambi	00°37.923N	030°14.643E	1558	55A
Kapchorwa	Binyinyi	-	01°25.042N	034°31.741E	1929	77A
Kisoro	Busanza	Nyakababi	01°10.066S	029°35.152E	1689	001A
Kisoro	Nyarubuye	Mwalo	01°15.127S	029°38.902E	1790	007A
Kisoro	Nyarubuye	Mwalo	01°15.127S	029°38.902E	1790	008A
Kisoro	Nyarusiza	Nyamushungwa	01°18.691S	029°40.856E	1985	012A
Kisoro	Nyarusiza	Buhangura	01°19.457S	029°40.987E	2045	016A
Lira	Amac	Acuma-roma	02°09.939N	033°01.885E	1071	56A
Lira	Amac	Adyeri	02°05.683N	033°02.392E	1060	57A
Lira	Amac	Corner Ariti	02°06.751N	032°59.934E	1060	59A
Maracha-Terego	Oluvu	Anderu	03°12.316N	030°51.911E	1221	64A
Maracha-Terego	Oluvu	Anderu	03°13.747N	030°50.747E	1238	65A
Maracha-Terego	Oluvu	Anderu	03°13.628N	030°51.030E	1254	66A
Maracha-Terego	Oluvu	Anderu	03°12.427N	030°52.328E	1203	67A
Maracha-Terego	Oluvu	Asuru	03°12.529N	030°51.891E	1212	69A
Maracha-Terego	Oluvu	Asuru	03°12.041N	030°50.730E	1240	71A
Maracha-Terego	Oluvu	Asuru	03°12.305N	030°51.912E	1228	72A
Maracha-Terego	Oluvu	Asuru	03°12.111N	030°52.182E	1218	73A
Mbale	Budwale	Bukumi	01°03.581N	034°15.572E	1862	91A
Mbale	Wanare	Bugeheme	01°03.158N	034°14.834E	1891	92A
Mbale	Wanare	Bugeheme	01°03.204N	034°14.618E	1871	94A
Mbale	Wanare	Bugeheme	01°03.451N	034°14.960E	1842	95A
Mbale	Budwale	Bukumi	01°03.520N	034°15.375E	1518	96A
Mbale	Wanare	Bugeheme	01°03.266N	034°14.982E	1854	97A
Mbale	Bungokho	Namashere	01°03.383N	034°13.907E	1187	98A
Mbale	Wanare	Bugeheme	01°03.197N	034°14.626E	1887	99A
Mbale	Budwale	Bukumi	01°03.621N	034°15.413E	1837	100A

Oyam	Loro	Alut-kot	02°13.793N	032°28.989E	1058	61A
Oyam	Loro	Adyeda	02°14.953N	032°33.087E	1051	62A
Oyam	Loro	Awe-ikwo	02°13.822N	032°29.041E	1061	63A
Sironko	Bukhalu	Bwerucra	01°17.537N	034°17.481E	1089	75A
Sironko	Bukhalu	Buyaga	01°16.478N	034°16.453E	1132	76A
Sironko	Masifwa	Jewa	01°10.236N	034°21.437E	1292	81A
Sironko	Masifwa	Lubenya	01°11.207N	034°22.697E	1549	82A
Sironko	Masifwa	Bugibulungi	01°09.068N	034°22.517E	1690	83A
Sironko	Masifwa	Bugimwera	01°11.472N	034°22.926E	1685	84A
Sironko	Masifwa	Gimbubuni	01°10.643N	034°22.584E	1485	85A
Sironko	Masifwa	Bunagani	01°10.529N	034°22.109E	1314	86A
Sironko	Masifwa	Lubenya	01°11.329N	034°22.810E	1690	88A
Sironko	Masifwa	Bunamono	01°09.173N	034°22.675E	1364	90A

51 *C. lindemuthianum* isolates that sporulated successfully. * A signifies Anthracnose

Diseased bean pod samples were collected from the sampled fields, placed in polythene bags and stored in boxes. A GPS machine was used to determine altitude and coordinates of fields where samples were picked.

3.2.2 Isolation of *C. lindemuthianum*

Isolation of the fungus was done according to the method described by Balardin et al. (1997). Infected tissues from the bean pods were cut into small pieces of up to 5cm long and the tissues were placed into a small beaker. 10ml of Sodium hypochlorite (Jik) bleach was added and after two minutes, the bleach was removed and 10ml of ethanol was added for two minutes before it was removed and the tissues rinsed with sterilized water. The tissues were placed on filter paper to remove excess water. The tissues were placed on PDA media and incubated in darkness at 22 - 24^{o}C for four days before sub-culturing onto modified Mathur's Agar media (500g) made up of 4g of Dextrose, 1.25g of Magnesium Sulfate, 1.35g of Potassium Phosphate, 1.2g of Neopeptone, 1g of Yeast extract and 8g of Agar, to get pure isolates and increase sporulation (Champion *et al*., 1973).

A second sub-culturing was done by placing single-spore isolates on fresh Mathur's agar (Plate 3.1) in a Petri dish and incubated at 22-24^{o}C for 7 to 10 days to allow the fungus enough time to produce conidial spores (Balardin *et al*., 1997). For inoculation purposes, conidial spores were scrapped off the growth medium into a small amount of water to make a suspension.

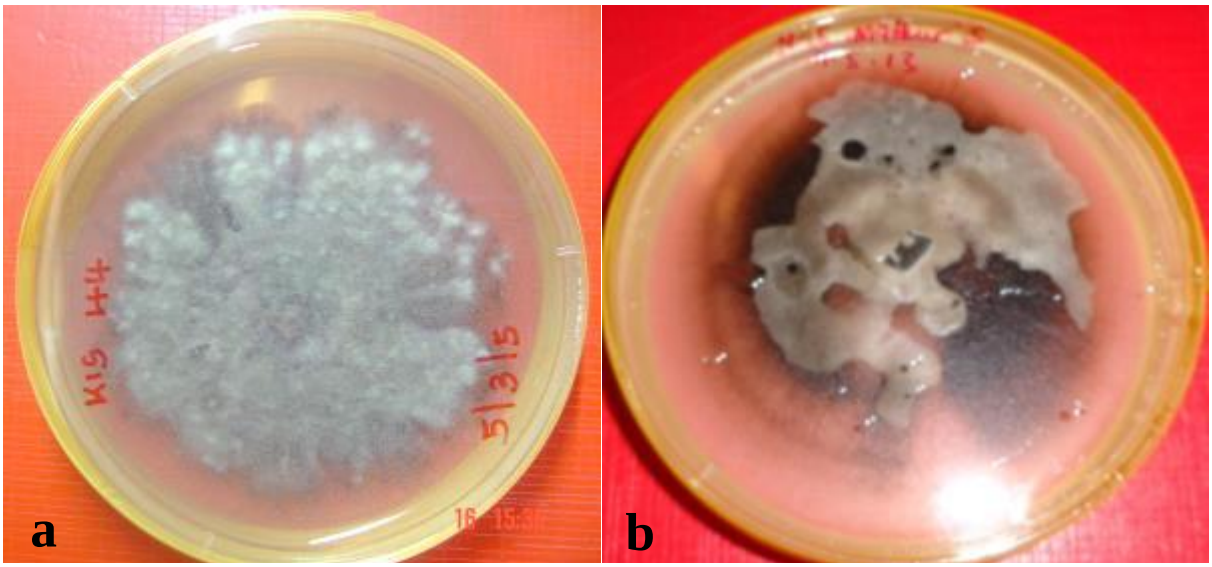

Plate: 3.1: *Colletotrichum lindemuthianum* in culture**; a =** Isolate Kis44 on PDA media; **b** = isolate N5 on Mathur's media;

3.2.3 Characterization of *C. lindemuthianum*

Inoculum was prepared by scrapping off germinated conidia (Plate 3.2) on the growth media in to a jar with small amounts of distilled water to form a suspension. The inoculum concentration was adjusted to 1.2 x 10^6 conidia ml^{-1} using a hemocytometer and 0.1% Tween 20 was added as a surfactant.

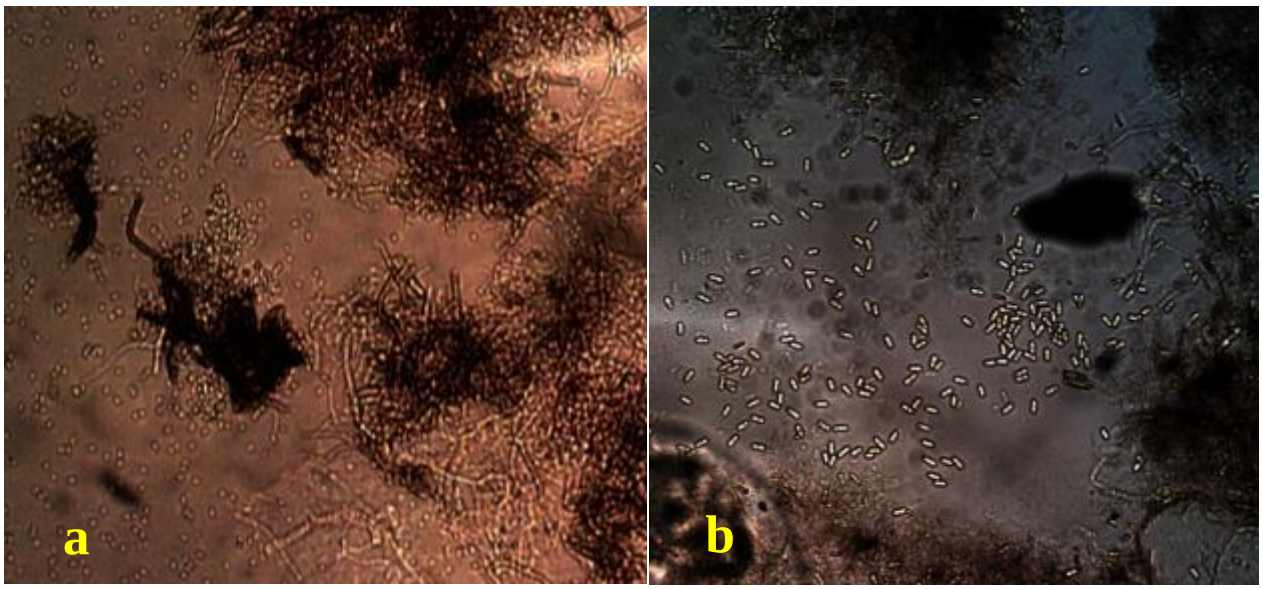

Plate 3.2: Conidia of *Colletotrichum lindemuthianum*; **a** = Conidia of isolate N2; **b** = Conidia of isolate Kis33A

Using a hemocytometer, the concentration was adjusted to 1.2 x 10^6 conidia ml^{-1} (Inglis *et al*., 1988) and 0.1% Tween 20 was added as a surfactant. Characterization of *C. lindemuthianum* was performed using a set of 12 differential bean cultivars (CIAT, 1988). Four of the differentials were large seeded varieties of Andean origin and the other six were small seeded middle-American genotypes (Table 3.2).

Table 3.2: Differential cultivars used to characterize *C. lindemuthianum, their b*inary codes, resistance genes and gene pool

Differential Cultivar	Seed Type [a]	Notation (n)	Binary Code (2^n)	Resistance Gene	Gene Pool [b]
Michelite	S	0	1	*Co-11*	MA
Michigan Dark Red Kidney	L	1	2	*Co-1*	A
Perry Marrow	L	2	4	*Co-1*^{3}	A
Cornell 49-242	S	3	8	*Co-2*	MA
Widusa	L	4	16	*Co-1*^{5}	A
Kaboon	L	5	32	*Co-1*^{2}	A
Mexico 222	S	6	64	*Co-3*	MA
PI 207262	S	7	128	*Co-4*^{3} *Co-9*	MA
TO	S	8	256	*Co-4*	MA
TU	S	9	512	*Co-5*	MA
AB 136	S	10	1024	*Co-6*	MA
G2333	S	11	2048	*Co-4*^{2} *Co-5*^{2} *Co-7*	MA

[a] S = Small seeded; L = Large seeded. [b] MA= Middle American; A= Andean ; Source: Awale et al., (2007).

Seed of the 12 differential cultivars were pre-germinated and later soaked for 30 minutes into the inoculum before transplanting into sterilized soil-saw dust composite in a controlled screen house. Five seeds of each differential cultivar and a susceptible check K132 were sowed in a tray. The humidity chamber conditions were maintained at a minimum of 95% relative humidity and temperature of 22±2^{o}C (Plate 3.3). Disease severity was scored 10 – 14 days after planting using a 1-5 scale (Inglis *et al*., 1988) where; 1 = no symptoms (resistant), 2-3 = very small lesions mostly on primary leaves (resistant) and 4-5 = numerous enlarged lesions or sunken cankers on the lower side of the leaves or hypocotyls (susceptible).

3.2.4 Race determination

To identify *C. lindemuthianum* races, the binary system of nomenclature (Pastor-Corrales, 1991) was used based on the sum of the binary values assigned to each of the 12 differential cultivars (Table 3.2) on which the unknown race is pathogenic. Each differential cultivar has an

assigned number 2^n where *n* corresponds to the place occupied by the cultivar within the differential series. The designation of a race number was obtained by summing the numerical values of all differential cultivars exhibiting susceptible (S) reactions to the isolate used for inoculation. Isolates with similar reactions on the differentials were grouped to form a race.

Plate: 3.3: Bean anthracnose symptoms on seedlings; **a** = Arrows pointing at anthracnose symptoms appearing on leaves of a susceptible cultivar; **b** = Arrows pointing at anthracnose symptoms on the cotyledon and hypocotyl of a susceptible cultivar

3.3 Results

3.3.1 Isolate and cultivar variability

The results of analysis of variance (Table 3.3) revealed highly significant differences (P≤0.001) among the cultivars and isolates.

Table 3.3: Analysis of variance for bean anthracnose severity on the 12 differential cultivars

	Severity		
Source of variation	**d.f**	**Mean square**	**% [a] Contribution**
Rep	4	1.10	1.51
Cultivar	12	33.78*	46.20
Isolate	50	36.41*	49.79
Cultivar x Isolate	600	1.33*	1.82
Residual	2,647	0.50	0.69

[a] Percentage contribution of each source of variation to the total variation
* Significant at P≤0.001

Variation due to the interaction between cultivars and isolates was also highly significant (P≤0.001). Isolate contributed the highest (49.8%) to the total observed variation followed by cultivars (46.2%).

The reactions of 51 isolates on the 12 differential cultivars are presented in the Table 3.4. The overall mean severity score was 2.64.

Table 3.4: Reactions of *Colletotrichum lindemuthianum* isolates on the differential cultivars

Isolate ID	Differential cultivars* and their resistance genes													Mean Sev
	1[b]	2[a]	3[a]	4[b]	5[a]	6[a]	7[b]	8[b]	9[b]	10[b]	11[b]	12[b]	13[ac]	
	Co-11	Co-1	Co-1[3]	Co-2	Co-1[5]	Co-1[2]	Co-3	Co-4[3], Co-9	Co-4	Co-5	Co-6, co-8	Co-4[2], Co-5[2], Co-7		
12A	1.80	4.20	2.40	1.40	2.00	2.00	2.40	1.40	1.40	1.80	3.20	1.40	2.00	**2.11**
34A	1.60	1.60	1.20	1.80	1.80	1.60	4.00	2.80	1.80	2.60	1.20	1.40	3.00	**2.03**
91A	3.00	2.80	3.20	2.00	2.40	2.00	1.80	2.20	1.60	1.40	2.60	1.00	2.80	**2.22**
92A	2.20	1.20	1.20	1.40	2.60	2.00	2.80	1.40	1.20	1.60	1.80	1.40	2.60	**1.80**
88A	2.20	2.60	3.00	1.40	2.20	2.80	3.40	2.20	2.40	1.80	2.00	1.20	2.20	**2.26**
65A	3.00	3.80	3.60	3.60	3.20	3.00	3.60	3.80	3.20	3.00	2.40	1.40	3.40	**3.15**
94A	1.80	2.40	2.20	2.00	2.40	2.20	2.00	1.60	2.60	1.40	2.40	1.00	2.60	**2.05**
56A	1.80	1.40	1.80	1.60	2.20	1.80	1.60	1.40	2.20	2.20	1.40	1.20	2.40	**1.77**
38A	2.00	1.40	1.60	1.40	1.40	1.40	1.40	2.00	1.60	2.00	1.60	1.20	1.80	**1.60**
08A	3.00	3.00	2.60	2.60	3.00	2.40	2.40	3.00	3.20	2.60	2.40	1.80	3.20	**2.71**
66A	3.20	3.60	3.20	3.00	3.60	2.80	3.80	3.60	3.20	3.00	2.80	2.20	3.60	**3.20**
90A	3.20	3.20	3.20	3.00	2.50	2.40	2.00	2.80	2.20	1.40	2.60	1.60	3.00	**2.55**
72A	2.40	1.60	2.00	1.80	2.20	1.60	1.40	2.60	2.00	1.40	2.60	1.20	2.60	**1.95**
64A	3.40	3.00	3.40	3.60	2.60	3.00	3.00	3.80	3.20	3.20	2.60	1.60	3.80	**3.09**
81A	2.00	2.40	1.80	2.20	2.00	2.40	1.00	1.80	2.60	2.40	2.20	1.00	3.00	**2.06**
100A	3.20	2.80	2.60	2.40	2.40	2.40	2.00	2.40	2.40	2.00	2.60	1.20	3.40	**2.45**
82A	2.20	1.60	2.00	1.40	2.00	1.80	1.80	1.20	2.00	1.20	1.60	1.00	2.20	**1.69**
59A	1.40	1.80	1.40	1.60	1.20	1.40	1.20	1.40	1.40	1.40	1.20	1.00	1.40	**1.37**
57A	1.40	1.20	1.60	1.40	1.80	1.40	1.40	1.40	1.60	1.20	1.60	1.00	1.60	**1.43**
63A	3.60	3.60	3.00	3.00	3.20	3.60	3.20	3.60	3.00	2.80	3.60	2.40	4.20	**3.29**
25A	1.20	1.40	1.20	1.20	1.40	1.00	1.00	1.20	1.20	1.20	1.20	1.00	1.40	**1.20**
44A	3.60	2.80	4.00	3.40	3.80	2.60	3.60	4.40	2.20	3.80	3.40	2.40	3.80	**3.37**
75A	3.00	3.20	2.80	3.40	3.40	3.40	3.20	3.80	2.20	3.00	2.80	2.00	4.20	**3.11**
84A	1.40	1.00	1.40	1.40	1.00	2.40	2.40	1.40	2.20	1.20	1.20	1.00	1.60	**1.51**
69A	2.80	3.00	3.00	2.60	3.60	3.20	3.20	3.00	2.00	3.40	1.60	1.00	3.80	**2.78**
41A	3.60	3.60	4.00	4.00	3.20	2.80	3.80	4.00	2.00	2.40	3.40	1.80	4.20	**3.29**
98A	3.00	2.60	3.20	2.80	2.60	3.20	3.00	2.80	3.00	2.60	3.20	1.60	3.80	**2.88**
52A	3.00	3.20	2.40	3.20	2.60	2.80	2.80	2.80	3.00	3.00	3.00	1.40	3.40	**2.82**

86A	2.60	2.00	2.00	2.60	1.60	1.80	2.40	2.20	1.60	2.20	1.80	1.20	2.60	**2.05**
67A	3.40	4.00	3.80	4.20	3.40	3.00	3.00	3.20	3.20	2.80	3.60	2.20	4.40	**3.40**
55A	4.00	3.60	3.20	3.80	3.00	3.40	3.60	4.00	3.80	3.80	2.80	1.80	4.00	**3.45**
95A	4.40	2.00	4.20	3.20	2.60	3.00	3.60	2.80	4.20	4.00	3.00	1.60	2.00	**3.12**
76A	4.40	4.20	4.20	4.80	4.60	4.20	3.80	4.20	4.60	4.20	4.20	2.60	4.00	**4.15**
28A	4.20	3.80	4.00	3.80	4.00	3.20	2.40	3.40	3.40	2.40	3.60	2.20	3.40	**3.37**
62A	4.60	2.20	2.40	2.80	3.00	3.00	3.00	2.80	3.40	4.00	1.60	1.00	3.40	**2.86**
37A	5.00	3.40	3.60	4.20	3.80	2.60	2.80	4.00	2.80	2.80	2.40	1.40	2.40	**3.17**
16A	3.60	2.60	3.60	1.80	2.60	3.40	2.20	3.60	4.20	1.40	3.00	1.00	3.40	**2.80**
83A	2.80	2.40	4.40	1.40	1.00	4.20	1.20	2.60	3.80	1.60	1.60	2.20	3.40	**2.51**
97A	3.20	2.60	4.20	1.00	2.60	4.40	2.40	3.40	2.80	2.60	4.60	1.80	3.20	**2.98**
99A	4.20	2.80	2.40	1.40	3.80	3.80	3.00	2.60	2.80	1.80	3.60	2.00	3.80	**2.92**
001A	4.80	4.40	4.60	4.60	4.80	4.40	4.60	5.00	4.40	4.80	5.00	3.20	4.60	**4.55**
77A	3.40	4.00	4.00	3.40	3.80	4.00	3.20	3.60	4.00	3.80	4.00	2.60	4.40	**3.71**
73A	3.40	4.00	4.00	3.40	3.80	4.00	3.20	3.60	4.00	3.80	4.00	2.60	4.40	**3.71**
46A	2.80	2.60	2.60	2.60	2.60	3.00	2.60	3.40	2.80	2.60	2.80	1.80	4.00	**2.78**
61A	2.00	2.40	2.00	2.00	2.00	2.00	1.60	2.20	2.20	1.80	2.00	1.20	3.40	**2.06**
40A	3.20	2.00	2.40	2.60	3.00	2.40	2.80	2.80	3.40	3.00	2.20	1.80	3.40	**2.69**
71A	2.80	2.40	3.40	3.60	3.00	3.60	3.00	3.60	2.60	2.80	3.00	1.60	4.40	**3.06**
007A	3.00	3.00	3.00	2.00	1.80	2.60	2.40	2.60	2.60	3.00	2.40	1.20	3.20	**2.52**
85A	1.80	2.20	2.00	2.40	1.60	2.60	2.00	1.20	1.20	1.60	1.40	1.00	3.40	**1.88**
36A	1.80	1.40	1.60	1.60	1.80	1.60	1.60	2.20	1.40	2.00	1.60	1.20	2.60	**1.72**
96A	3.00	3.20	3.40	3.60	3.40	3.20	3.40	3.20	3.20	2.80	3.20	2.20	4.00	**3.22**
Mean Sev	**2.91**	**2.69**	**2.82**	**2.58**	**2.66**	**2.72**	**2.61**	**2.78**	**2.65**	**2.48**	**2.58**	**1.58**	**3.19**	**2.64**
S.e	**0.13**	**0.13**	**0.13**	**0.14**	**0.13**	**0.12**	**0.12**	**0.14**	**0.13**	**0.13**	**0.13**	**0.08**	**0.12**	**0.10**

*****Differential cultivar; 1 = Michelite; 2 = MDRK; 3 = Perry Marrow; 4 = Cornell 49-242; 5 = Widusa; 6 = Kaboon; 7 = Mexico 222; 8 = PI 207262; 9 = TO; 10 = TU; 11 = AB136; and 12 = G 2333

[a] Andean gene pool; [b] Mesoamerican gene pool; [c] Susceptible local check

Mean severity scores on cultivars across 51 isolates ranged from 1.58 to 3.19. The cultivar G2333 had the lowest mean score (1.58) followed by cultivars Tu (2.48), Cornell 49-242 (2.58) and AB136 (2.58), while the susceptible check K132 had the highest mean severity score (3.19). The isolate 001A had the highest mean severity (4.15) across cultivars followed by isolates 76A (4.15), 77A (3.71), 73A (3.71) and 67A (3.40), while the isolate 25A had the lowest mean severity (1.20) across cultivars.

3.3.2 Race determination

Results of race determination through screening of bean differential cultivars are presented in Table 3.5. Using the binary system, twenty seven (27) races were identified out of the 51 isolates. Races 2047 and 4095 were the most abundant.

Table 3.5 Characterization of *C. lindemuthianum* pathotypes using differential cultivars

	Differential cultivars[1] and their respective resistance genes [a]												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
Isolates	**Co-11**	**Co-1**	**Co-1[3]**	**Co-2**	**Co-1[5]**	**Co-1[2]**	**Co-3**	**Co-4[3], Co-9**	**Co-4**	**Co-5**	**Co-6, co-8**	**Co-4[2], Co-5[2], Co-7**	**Race***
38A, 59A, 57A, 25A	R	R	R	R	R	R	R	R	R	R	R	R	0
82A	S	R	R	R	R	R	R	R	R	R	R	R	1
85A	R	S	R	S	R	S	R	R	R	R	R	R	42
92A	S	R	R	R	S	R	S	R	R	R	R	R	81
36A	R	R	R	R	R	R	R	S	R	R	R	R	128
84A	R	R	R	R	R	S	S	R	S	R	R	R	352
61A	R	S	R	R	R	R	R	S	S	R	R	R	386
88A	S	S	S	R	S	S	S	S	S	R	R	R	503
34A	R	R	R	R	R	R	S	S	R	S	R	R	704
86A	S	R	R	S	R	R	S	S	R	S	R	R	713
69A	S	S	S	S	S	S	S	S	R	S	R	R	767
56A	R	R	R	R	S	R	R	R	S	S	R	R	784
62A	S	S	S	S	S	S	S	S	S	S	R	R	1023
12A	R	S	S	R	R	R	S	R	R	R	S	R	1094
72A	S	R	R	R	S	R	R	S	R	R	S	R	1169
91A	S	S	S	R	S	R	R	S	R	R	S	R	1175
94A	R	S	S	R	S	S	R	R	S	R	S	R	1334
90A, 100A	S	S	S	S	S	S	R	S	S	R	S	R	1471
16A, 99A	S	S	S	R	S	S	S	S	S	R	S	R	1527
41A	S	S	S	S	S	S	S	S	R	S	S	R	1791
81A	R	S	R	S	R	S	R	R	S	S	S	R	1834
007A	S	S	S	R	R	S	S	S	S	S	S	R	2023
97A	S	S	S	R	S	S	S	S	S	S	S	R	2039
95A, 40A	S	R	S	S	S	S	S	S	S	S	S	R	2045
65A, 08A, 64A, 75A, 98A, 52A, 55A, 37A, 46A,71A	S	S	S	S	S	S	S	S	S	S	S	R	2047
83A	S	S	S	S	R	S	R	S	S	R	R	S	2479
66A, 63A, 44A, 67A, 76A, 28A, 001A, 77A, 73A, 96A	S	S	S	S	S	S	S	S	S	S	S	S	4095

*Races characterized using the binary system (Pastor Coralles, 1991); R=Resistant reaction; S=Susceptible reaction

[1] Position numbers and Binary codes: Michelite (0)1; MDRK (1)2; Perry Marrow (2)4; Cornell 49-242 (3)8; Widusa (4)16; Kaboon (5)32; Mexico 222 (6)64; PI 207262 (7)128; TO (8)256; TU (9)512; AB136 (10)1024; and G 2333 (11)2048.

Race 4095 was the most virulent causing a susceptible reaction on all the 12 differential cultivars and the susceptible check. This was followed by races 2047, 2045 2039 and 2023. Race 2047 comprised of 10 isolates collected from five districts namely Kabarole, Kisoro, Maracha, Mbale and Sironko.

3.3.3 Race distribution

Race 2479 comprised of one isolate collected from Sironko district, while race 4095 comprised of 10 isolates collected from seven districts of Kabarole, Kapchorwa, Kisoro, Oyam, Mbale, Maracha and Sironko making race 4095 the most widely distributed followed by race 4027. Sironko and Mbale districts had the highest number of races including the most virulent ones followed by Kabarole district and Kisoro district. These districts are all in high altitude bean growing regions ranging from 1429 – 1860m above sea level and therefore offer favorable conditions for the development and spread of bean anthracnose disease. Results of incidences and severity of bean anthracnose in the sampled districts are presented in Table 3.6.

Table 3.6: Incidence, Severity and races of *C. lindemuthianum* by district

District	Incidence (%)	Severity (1-5)	Cultivars sampled	Races identified
Lira	23	2.3	Variety mixtures (3)	0, 784
Sironko	76	4.3	Unknown local varieties (10)	1, 42, 352, 503, 713, 1471, 1834, 2047, 2479, 4095
Mbale	75	4.0	Unknown local varieties (9)	81, 1175, 1334, 1471, 1527, 2039, 2045, 2047, 4095
Oyam	31	1.5	Variety mixtures (2) Bweyale-yellow	1023, 4095 386
Maracha	45	2.6	Ofuta ofuta, Agrupia, Wande-wande, Mvugupia, K132	2047,4095 4095 767 1169
Zombo	0	0.0	Cogudibi, Lau lau, Mixed varieties, Nyar adranga, Ocidu, Nyar awora	- - -
Kabarole	-	-	Variety mixtures (12)	0, 128, 704, 1791, 4095, 2045, 2047
Kisoro	-	-	Variety mixtures (5)	1094, 1527, 2023, 2047, 4095

Sironko district had the highest incidence (76%) and severity (4.3), followed by Mbale (75%, 4.0), Maracha (45%, 2.6) in West Nile and Oyam (31%, 1.5) in the North. Most of the varieties sampled were local or variety mixtures. K132 was the only improved variety observed in Maracha out of all the districts sampled. Zombo district had no incidence (0.0).

The pathogenicity of the 27 races on the 12 differential cultivars is presented in Table 3.7.

Table 3.7: Pathogenicity of 27 races on 12 bean differential cultivars and a susceptible check

Differential Cultivar	Resistance genes/ alleles possessed	Number of races with a resistant reaction	Mean severity	Cultivar rank*
G2333	*Co-4^2, Co-5^2, Co-7*	25	1.58±0.08	1
Cornell 49-242	*Co-2*	16	2.58±0.14	2
TU	*Co-5*	15	2.48±0.13	3
AB136	*Co-6, co-8*	14	2.58±0.13	4
Mexico 222	*Co-3*	12	2.61±0.12	7
TO	*Co-4*	12	2.65±0.12	8
Widusa	*Co-1^5*	12	2.66±0.13	6
Perry Marrow	*Co-1^3*	12	2.82±0.13	5
Kaboon	*Co-1^2*	11	2.72±0.12	9
MDRK	*Co-1*	10	2.69±0.13	11
Michelite	*Co-11*	10	2.91±0.13	10
PI207262	*Co-4^3, Co-9*	9	2.78±0.14	12
K132 (check)	-	7	3.19±0.12	13

* Ranking was based on number of races with resistant reaction on any given cultivars

The differential cultivar G2333 was resistant to 25 out of the 27 races, which was the highest number of resistant reactions by a single cultivar. This was followed by cultivars Cornell 49-242, TU, AB 136 and Mexico 222.

3.4 Discussion

The results revealed a high level of pathogenic variability of *C. lindemuthianum* with 27 races identified from 51 isolates collected from major bean growing districts in Uganda (Table 3.5). Mwesigwa (2008), using differential cultivars, similarly reported a high level

of variability of *C. lindemuthianum* in Uganda with 21 races identified from 47 isolates which grouped into three subgroups using RAPD.

Only three races in this study were similar to races reported earlier in Uganda namely race 0 and 128 (Mwesigwa, 2008) and race 767 (Nkalubo, 2006), which was reported as the most abundant and aggressive while the previously reported aggressive races were 1024, 1536, 1538, 1856, 1857, 1989, 3086 and 4033 (Mwesigwa, 2008). This study, however, revealed that races 4095, 2047, 2045, 2039 and 2023, which had not been reported before were the most aggressive (Table 3.5). This lack of consistency may be attributed to differences in areas sampled and/ or mutation of the pathogen to produce new pathotypes. The Race 2047 was reported by de Lima Castro et al. (2017) as a Mesoamerican race. It is reported in Brazil to be one of the most aggressive of *C. lindemuthianum* races that can overcome anthracnose resistance conferred by seven resistance genes namely *Co-1, Co-2, Co-3, Co-4, Co-5, Co-6* and *Co-11*; and five alleles namely $Co\text{-}1^2$, $Co\text{-}1^3$, $Co\text{-}1^5$, $Co\text{-}3^3$ and $Co\text{-}4^3$ (Darben *et al*., 2017). Mahuku and Riascos (2004) isolated race 2047 in Colombia from materials obtained from Costa Rica.

The anthracnose disease was not observed in Zombo district of West Nile despite the fact that it is a highland locale with favorable conditions for the disease to flourish. This could be due to the limited use of seed from other regions since bean growers in the district mostly rely on mixed seed of local varieties. This limits the possibility of anthracnose spread through movement of seed. Absence of the disease could also be due to sampling in first season (March – June), which has less rain and humidity in the West Nile zone than the second season (August to November) (http://Weatherspark.com)

The differential cultivar G2333, Cornell 49-242, TU and AB 136 respectively showed the most broad-spectrum resistance against *C. lindemuthianum* races in Uganda (Table 3.7) implying that the genes $Co\text{-}4^2$, $Co\text{-}5^2$, *Co-7* from cultivar G2333, *Co-2* from cultivar Cornell 49-242, *Co-5* from cultivar TU and *Co-6, co-8* from cultivar AB136 are the best resistance genes against bean anthracnose disease in Uganda. Bigirimana et al., (2000) reported the cultivars TU, AB136 and G2333 to be highly resistant against 12 isolates from major bean growing areas in Burundi. Nkalubo (2006) also reported cultivars

G2333 and AB136 as the most resistant. However, Mwesigwa (2008) reported cultivar Widusa (*Co-1*^{5}), which did not succumb to any of the 41 isolates, as the most resistant followed by G2333. Clearly the cultivars G2333, Cornell 49-242, TU and AB 136 stand out as the most resistant and would be the best choices to use as donor parents in a breeding program aiming at increasing spectrum and durability of resistance against bean anthracnose. The highly effective resistance in cultivar G2333 is mostly attributed to its naturally occurring pyramid comprising of *Co-4*^{2}, *Co-5* and *Co-7* genes (Young *et al.*, 1998). The allele *Co-4*^{2} is recognized as being among the most effective resistance genes against *C. lindemuthianum* described in common beans (Silverio *et al*., 2002).

It should be noted that all the six genes above are from the Mesoamerican gene pool. When considering gene pyramiding for more effective resistance to *C. lindemuthianum* it is recommended to combine resistance genes from both the Mesoamerican and Andean gene pools (Kelly *et al*., 1994). The Andean gene *Co-1* is multiple allelic having four different alleles namely *Co-1, Co-1*^{2}, *Co-1*^{3} & *Co-1*^{5} from cultivars Michigan Dark Red Kidney, Kaboon, Perry marrow and Widusa respectively. Based on results of this study the alleles *Co-1*^{3} & *Co-1*^{5} were more effective and therefore, any of them may be combined in pyramid with the above Mesoamerican genes for a more durable resistance against *C. lindemuthianum* in Uganda.

It was observed, however, that, G2333 succumbed to two races namely 2479 and 4095. Mwesigwa (2008) also reported two races 3086 and 4033 that caused symptoms on cultivar G2333. The cultivars Widusa (*Co-1*^{5}), Mexico 222 (*Co-3*), Tu (*Co-5*) and AB136 (*Co-6, co-8*) showed resistance to race 2479, implying that any of the genes in these cultivars could be combined with the genes in cultivar G2333 for form new highly effective pyramids. Although, Leaky and Simbwa-Bunnya (1972) observed an immune nature of resistance in the cultivar Cornell 49-242 with a single dominant gene *Co-2*, this study showed that this cultivar had anthracnose symptoms with 11 races. It still ranked second after G2333 in conferring resistance to *C. lindemuthianum* in Uganda

The cultivars Michelite (*Co-11*), MDRK (*Co-1*) and PI207262 (*Co-4*^{3}, *Co-9*) had the lowest number of resistant reactions implying that the resistance genes they carry are less

broad-spectrum. These genes could still be useful in breeding programs targeting resistance gene pyramiding for broad spectrum resistance and/ or specific resistance to races 0, 42, 128, 352, 386, 704, 784, 1094, 1334 and 1834 for cultivar Michelite; and races 0, 1, 42, 81, 352, 784, 1094, 1334 and 1834 for cultivar PI207262. The cultivar PI207262 was the most susceptible to *C. lindemuthianum* rather than the earlier reported cultivar Michelite. Mwesigwa (2008) observed the same about Michelite, which is usually reported as a universal susceptible differential cultivar. The resistance in Michelite is attributed to the resistance gene designated as *Co-11* (Gonçalves-Vidigal *et al*., 2007), which appears to have some level of resistance to *C. lindemuthianum* races in Uganda. Bigirimana et al. (2000) ranked the cultivar PI207262 second lowest after Michelite out of the 12 differential cultivars. The cultivar PI207262 containing a natural resistance gene pyramid *Co-4*^{3}*+Co-9* possesses a very specific breeding value against Andean races of *C. lindemuthianum* and is recommended for use only to diversify the resistance in new gene pyramids (Kelly and Vallejo, 2004).

3.5 Conclusion

Colletotrichum lindemuthianum showed a high pathogenic variation in Uganda with 27 races identified from the sampled bean crops growing areas and pathogenic variation was highest in the Eastern and South Western highland regions of Uganda. Races 2479 and 4095 were the most aggressive causing a susceptible reaction on the most resistant cultivar G2333 among the 12 differential cultivars, implying existence of new and highly aggressive races in Uganda and the urgent need for enhancing efforts towards management and control of bean anthracnose disease.

3.6 Recommendations

The Cultivars G2333, Cornell 49-242, TU and AB136 are recommended for use as sources of broad-spectrum resistance to be introgressed into susceptible cultivars. Further studies should be conducted to investigate and identify other sources of effective resistance against *C. lindemuthianum*; use of molecular tools to understand the genetic variability of *C. lindemuthianum*; and assess effectiveness of the single genes *Co-4*^{2}*, Co-5*^{2}*, Co-5, Co-1*^{3}*, Co-1*^{5}*, Co-3, Co-6, co-8* and behavior in pyramid with each other against *C. lindemuthianum* in Uganda.

CHAPTER FOUR

Effectiveness of pyramided resistance genes to anthracnose in common bean populations

Abstract

Bean anthracnose is one of the most devastating diseases that constrain common bean production in Uganda. This study investigated the effectiveness of pyramided and single genes. SCAR markers were used to facilitate the process of pyramiding three anthracnose resistance genes using a cascading pedigree pyramiding scheme. Detached leaf trifoliates of 40 $F_{4:6}$ families were screened with four *C. lindemuthianum* races and severity scored using Balardin et al (1997) scale. Disease severity data were subjected to ANOVA. Correlation and path analysis were done to establish relationships between number of pyramided genes and the yield traits. Races, genotypes and Race x Genotype interaction were all significant ($P<0.001$). *Co-4*^{2}*+Co-5+Co-9* and *Co-4*^{2}*+Co-5* pyramids had the lowest severity scores. The group *Co-4*^{3}*+Co-9* had the highest severity score. The *Co-4*^{2} and *Co-5* genes conferred resistance to all the four races 352, 713, 767 and 2047. The single gene *Co-4*^{2} was not significantly different from the best pyramids *Co-4*^{2}*+Co-5+Co-9* and *Co-4*^{2}*+Co-5* (*P<0.01*). Similarly the *Co-5* gene was not significantly different from *Co-4*^{2}*+Co-5, Co-4*^{2}*+Co-9* and *Co-5+Co-9* pyramids (*P<0.01*). The *Co-9* gene showed antagonism in all pyramids. Number of pyramided genes negatively correlated with seed weight per plant (-0.17), number of pods per plant (-0.24, $p<0.05$) and number of seeds per plant (-0.19, $p<0.1$) and had a significant ($P<0.05$) negative indirect effect (-0.25) on seed weight per plant via number of seeds per plant. Therefore, the effectiveness of pyramided genes highly depended on the individual genes combined. The single genes *Co-4*^{2} and *Co-5* had factors that promote broad-spectrum resistance to *C. lindemuthianum*. Pyramiding of resistance genes had negative direct and indirect effects on yield traits.

Key words: SCAR markers, *Colletotrichum lindemuthianum*, broad-spectrum resistance

4.1 Introduction

Colletotrichum lindemuthianum has a high degree of pathogenic and genetic variability (Mahuku and Riascos, 2004) and new races that break down resistance in commercial cultivars continually emerge (Kelly *et al*., 1994). In many cases, commercial cultivars are resistant to some races but not to others (CIAT, 1997) leading to breakdown of resistance. This has made single gene deployment as a strategy to control bean anthracnose disease, ineffective.

Gene pyramiding is a technique aimed at combining multiple desirable genes from multiple parents into a single genotype (Ye and Smith, 2008). Using this technique, it is possible to combine more than one anthracnose resistance genes into a susceptible bean variety. Conventional gene pyramiding, however, is a long and costly process (Joshi and Nayak, 2010) requiring extensive phenotyping with several races of the pathogen for many generations (Ye and Smith, 2008). In addition, different loci may be involved in the genetic control of a resistant phenotype, and the expression of specific genes can be masked due to epistatic interactions (Ferreira *et al*., 2012).

The development of modern plant molecular techniques increased the applicability of gene pyramiding because marker based selection reduces extensive phenotyping, thus reducing the breeding time and cost (Ye and Smith, 2008). Moreover, DNA markers greatly facilitate selection of plants with desired traits allowing selection for multiple specific genes from one DNA sample without phenotyping (Collard and Mackill, 2008). Marker assisted selection (MAS) has, therefore, been extensively used in common bean breeding (Miklas *et al*. 2006) and molecular markers linked to the majority of major anthracnose (*Co*-) genes were reported and provide an opportunity to enhance disease resistance through MAS (Kelly & Vallejo, 2004).

Different authors have recommended the use of gene pyramiding as a strategy to increase broad-spectrum resistance to bean anthracnose. Kelly et al. (1994) recommended pyramiding *Co-1* Andean and *Co-2* Mesoamerican genes against all known *C. lindemuthianum* races in North America. Young and Kelly (1996) suggested pyramiding

of major genes such as *Co-6* and *Co-5* in combination with the *Co-1* gene for durable resistance against anthracnose in common beans. For successful resistance gene pyramiding, however, there is need to understand the nature of resistance conferred by single genes and the different pyramided gene combinations under a diverse *C. lindemuthianum* population. The purpose of this study therefore was to; a) develop advanced bean populations/ lines with pyramided anthracnose resistance genes; and b) to assess the effectiveness of single and pyramided resistance genes in conferring broad resistance to diverse *C. lindemuthianum* races.

4.2 Materials and methods

4.2.1 Parent materials and locations for breeding

All the cultivars used as parents were obtained from the Legumes Program, National Crop Resources Research Institute (NaCRRI), Namulonge, located 00 32" N of the Equator and 320 37" E, 27Km North of Kampala and elevated at 1,150 meters above sea level (asl). All crosses and advancement from F_1 to F_4 generations were conducted under screen house conditions at NaCRRI. Marker Assisted Selection (MAS) for allele selection during gene pyramiding, fixation of alleles from F_4 up to F_6, genotypic and phenotypic screening of advanced lines were conducted at the International Center for Tropical Agriculture (CIAT), based at the National Agricultural Research Laboratories (NARL), Kawanda, located 0^o 24' 38.15" N and 32^o 32' 14.06" E and elevated at 1,147 meters above sea level. The Parents and their traits used in this study are indicated in Table 4.1.

Table 4.1: Parents used in the gene pyramiding scheme

Cultivar	Pedigree	Growth habit	Gene pool	Anthracnose Resistance genes	Response to anthracnose
K132[a]	Calima-2 x Argentino 1	B	A	-	Susceptible to anthracnose (Nkalubo *et al.,* 2009)
NABE 4[a]	Sug 47 x Cal 103	B	A	-	Susceptible to anthracnose (Mwesigwa, 2008)
NABE 13[a]	RWR 1946	B	A	-	Susceptible to anthracnose
NABE 14[a]	RWR 2075	B	A	-	Susceptible to anthracnose

G 2333[a,b]	Colorado de Teopisca	C	MA	*Co-4*^{2}, *Co-5* & *Co-7*	Resistant to anthracnose (Kelly and Vallejo, 2004)
PI 207262[c]	Tlalnepantla 64	C	MA	*Co-4*^{3} & *Co-9*	Resistant to some races of anthracnose (Kelly and Vallejo, 2004)
RWR719[d]	unknown	B	MA	-	Donor for Pythium root rot resistance (Namayanja *et al.*, 2014)

[a] Officially released varieties in Uganda
[b] Landrace from Mexico, released in the great lakes region as Umubano and NABE10C
A = Andean; MA = Mesoamerica; B = Bush; C = Climber

4.2.2 Development of populations

A cascading pedigree gene pyramiding scheme (Servin, *et al*., 2004), was used to develop populations with pyramided resistance genes against bean anthracnose. In this breeding scheme, only one cross was made at each generation beginning with two founding parents and followed by an intermediate genotype and one founding parent. The minimum population size to ensure at a predetermined probability of 95% or 99%, that at least one desired genotype is present in a population was derived from the equation; N_q = In (1-*q*)/ In(1-*p*) (Ye and Smith, 2008) where; *N* = minimum population size, *p* = number of individuals of the desirable genotype in a population of size *N* and *q* = predetermined probability.

A root genotype is an intermediate genotype in which all the desired genes have been introgressed but still in heterozygous state (Servin, *et al*., 2004). After obtaining the root genotype, the fixation of these genes was achieved through selfing.

4.2.2.1 Selection scheme

Pedigree breeding method was used to advance selected individuals that possessed the desired number and combination of resistance genes. Marker Assisted selection (MAS) was used to select individuals with the desired single and pyramided genes during the pedigree and fixation steps of the breeding scheme.

4.2.2.2 Molecular markers used in MAS

The target genes, linked DNA markers used to tag the genes, their sizes, orientation and primer sequences are presented in Table 4.2.

Table 4.2: PCR-based markers used in Marker Assisted Selection

Marker	Locus/ Gene tagged	Original marker*	Linkage group	Size (bp)/ orientation	Primer Sequence	Reference
SBB14	*Co-4*^{2}	BB14	B8	1150/1050, codominant	*Forward:* GTG GGA CCT GTT CAA TAA TAC *Reverse:* GTG GGA CCT GGG TAG TGT AGA AAT	Awale and Kelly (2001)
SAS13	*Co-4*	AS13	B8	950, *Cis*	*Forward:* CAC GGA CCG AAT AAG CCA CCAACA *Reverse:* CAC GGA CCG AGG ATA CAG TGA AAG	Young et al. (1998)
SAB3	*Co-5*	AB-3	B7	400, *Cis*	*Forward:* TGG CGC ACA CAT AAG TTC TCA CGG *Reverse:* TGG CGC ACA CCA TCA AAA AAG GTT	Vallejo and Kelly, (2001)
SB12	*Co-9*	B-12	B4	350, *Cis*	*Forward:* CCT TGA CGC ACC TCC ATG *Reverse:* TTG ACG ATGGG TTG GCC	Mendez de Vigo et al. (2002)
PYAA19$_{800}$	*Prr*	AA19	-	800, *Cis*	Forward: TTA GGC ATG TTA ATT CAC GTT GG Reverse: TGA GGC GTG TAA GGT CAG AG	Muhuku et al. (2007)

*Original markers were Random Amplified Polymorphic DNA (RAPDs) markers converted to SCARs

Sequence Characterized Amplified Regions (SCAR) markers were used to tag and track the resistance genes of interest. The primers were obtained from the Department of Molecular and Cellular Biology, University of Cape Town, Randebosch, South Africa. Later batches in premix form were obtained from Bioneer Corporation, Munpyeong-dong, Daejeon, South Korea. A 25/100 base pairs (bps) mixed DNA molecular weight marker (Ladder), specifically designed for determining the size of double strand DNA from 25 to 2,000 bps, was used.

4.2.2.3 DNA extraction and storage

Leaf samples were picked from 14-day old plants raised in a screen house. Genomic DNA was extracted using the Cetyltrimethylammoniumbromide (CTAB) method adapted from Doyle and Doyle (1987). 50-mg samples of young leaf tissues were ground to a fine powder in liquid nitrogen. The powder was placed in labelled 1.5mls eppendorf microtubes. 500µl of 2% CTAB extraction buffer [20 mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, 2% CTAB, plus 0.4% beta-mercaptoethanol added just before use] was added to the microtubes containing the crushed leaf samples. The solution was incubated at 55ºC for 45 min while gently mixing by inversion every 15 min. 500 µl of chloroform-isoamylalcohol (24:1) was added to the tubes and gently mixed for one (1) minute. Samples were centrifuged for 10 minutes at 12,000 revolutions per minute (rpm). 600 µl of the supernatant was then transferred to a fresh labelled tube following the addition of 500 mL chloroform-isoamylalcohol (24:1). This procedure was repeated twice. 500 mL of the supernatant was then transferred to a fresh tube with 700 µl of cold isopropanol (-20ºC). Samples were gently mixed by inversion and centrifuged at 12,000 rpm for 10 minutes making it possible to visualize the DNA adhered to the bottom of the tube. The liquid solution was then released and the DNA pellet washed with 700 µl of 70% ethanol to eliminate salt residues adhered to the DNA. The DNA was left to dry for approximately 12 hours with the tubes inverted over a filter paper, at room temperature. The DNA pellet was then re-suspended in 100µl TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0) plus 5µl ribonuclease (RNAse 10 mg mL–1) in each tube. The DNA solution was incubated at 37ºC for 1h and thereafter stored at -20ºC.

4.2.2.4 DNA amplification, gel electrophoresis and imaging

The reagents used for preparing the Polymerase Chain Reaction (PCR) master mix, their volumes and concentrations are presented in Table 4.3.

Table 4.3: Ingredients of a PCR master mix using PCR reagents and a Bioneer PCR premix

PCR Reagents	Reaction Volume (µl)	Stock concentration	Final concentration
ddH_2O	10.6		
dNTPs	0.8	10mM	0.1mM
Primer 1	1.0	10mM	0.1µM
Primer 2	1.0	10mM	0.1µM
$MgCl_2$	1.2	50mM	1.5mM
PCR Buffer	2.0	10X	1X
Taq Polymerase enzyme	0.4	5U/µl	0.2U
PCR Premix			
ddH_2O	17		
Primer 1	1.0	10mM	0.1µM
Primer 2	1.0	10mM	0.1µM

To prepare a PCR master mix using PCR reagents, double distilled water (ddH_2O), deoxy nucleoside triphosphates (dNTPs including dATP, dGTP, dCTP and dTTP), forward and reverse primers, $MgCl_2$, PCR buffer and *Taq* polymerase enzyme were mixed based on the concentrations in Table 4.3 and the reaction volumes were multiplied by the number of samples to be amplified to get to total master mix volume. Nineteen microliters (19µl) of the master mix were pipetted into individual PCR tubes and 1.0µl of 40ng plant DNA were added to each tube to make a total PCR reaction volume of 20µl.

When the PCR Bioneer Premix (Bioneer Inc, Korea) was used instead of the PCR reagents, the PCR master mix was prepared by adding ddH_2O to a 1.5ml Eppendorf tube, followed by the forward and reverse primers according to the working concentrations in Table 4.3. The total master mix volume was derived by multiplying the individual reaction volumes of the ingredients by the number of samples to be amplified. Nineteen (19) microliters were pipetted into the PCR tubes containing the premix pellet, which is made up of 2mM $MgCl_2$, 0.2mM dNTPS, 0.2U Top DNA polymerase and a tracking dye. One microliter (1.0µl) of 40ng DNA was added to make a total reaction volume of 20µl. Amplification of DNA was carried out in a Bioneer Thermal cycler (Bioneer Inc, Korea)

with an initial denaturation step at 95^{o}C for 5 minutes and 35 cycles each of a denaturation step at 94^{o}C for 20 seconds, an annealing step at 64^{o}C (*SBB14*), 68^{o}C (SAS13), 65^{o}C (*SAB3*), 65^{o}C (*SB12*), 64^{o}C (*SH18*) and 63^{o}C ($PYAA19_{800}$) for 40 seconds; an extension step at 72^{o}C for one (1) minute followed by a final extension for 10 minutes at 72^{o}C. The amplicons were resolved on 1.5% agarose gels in 1X TBE (0.045 M Tris–borate and 1 mM EDTA, pH 8.2) at 100V for 90 minutes and stained with 0.5µg/ml ethidium bromide for 10 minutes. Gel images were captured using the SynGene G: BOX gel documentation system (Syngene, Frederick, MD, USA).

4.2.2.5. Pedigree steps of the pyramiding scheme

The donor parents for anthracnose resistance G2333 and PI207262 were crossed in a screen house to combine the target anthracnose resistance alleles *Co-4^{2}/Co-4^{3}*, *Co-5*, *Co-9* in F_{1a} plants (G2333 x PI207262). The F_{1a} plants were crossed with RWR719 to produce F_{1b} plants [(G2333 x PI207262) x RWR719]. The F_{1b} seeds of the cross were planted as single potted plants along with varieties K132, NABE4, NABE13 and NABE14. The SCAR markers *SAS13*, *SBB14*, *SAB3* and *SB12* were run on extracted DNA of 105 F_{1b} plants to identify plants that possessed all the target genes before crossing with the susceptible varieties. DNA of 35 F_{1b} plants positively amplified all the three desired anthracnose resistance genes (*Co-4^{2}/Co-4^{3}*, *Co-5* and *Co-9*). Six (6) of the 35 F_{1b} plants were selected and crossed with plants from each of the four recurrent parents from the susceptible varieties. F_{1c} seeds of the crosses [((G2333 x PI207262) x RWR719) x K132, NABE4, NABE13, NABE14] were harvested and replanted as individual plants under screen house conditions. Markers *SBB14*, *SAB3*, *SB12* and $PYAA19_{800}$ were run on DNA extracted from 46 F_{1c} plants. Three plants possessing all three anthracnose resistance genes were identified. These plants possessing all desired genes in heterozygous state became the root genotype (Servin *et al*., 2004).

4.2.2.6 Fixation steps of the pyramiding scheme

F_{1c} seed of the root genotype from the cross [((G2333 x PI207262) x RWR719) x K132, NABE4, NABE13, NABE14] were sowed and raised under screen house conditions and allowed to self-fertilize. F_2 seed from the root genotype was replanted and DNA extracted from 69 F_2 plants. The DNA was amplified and MAS conducted using *SBB14*, *SAS13*,

SAB3, SB12 and *PYAA19$_{800}$* SCAR markers to identify and categorize F_2 plants that inherited 0, 1, 2, 3 or 4 resistance genes.

Segregating plants were categorized based on number of resistance genes inherited and based on the recurrent parent. From this point forward plants were advanced based on the two categories in order to have advanced genotypes with varying numbers of fixed resistance genes in the genetic background of the four recurrent parents K132, Nabe 4, Nabe 13 and Nabe 14.

DNA of 156 F_3 plants was amplified using *SBB14, SAS13, SAB3, SB12* and *PYAA19$_{800}$* SCAR markers and out of these six plants inherited all the four target genes. Selfing continued up to F_6 generation to ensure that the genes were fully fixed in homozygous state but MAS was conducted up to F_4 generation of the fixation scheme.

4.2.3 Raising the bean populations for phenotypic screening

Advanced lines at F_6 stage were used for phenotypic screening. Seed of selected F_6 lines was sown in five liter plastic pots filled with top soil mixed with sand and sterilized manure in the ratio of 5:3:2 respectively under greenhouse conditions. Diammonium Phosphate (DAP) fertilizer was applied prior to sowing and watering was done daily.

4.2.4 Phenotypic screening for bean anthracnose resistance

The F_6 advanced lines were grouped into 10 groups according to the number and type of gene inherited as indicated in Table 4.4. Four (4) *C. lindemuthianum* races (Plate 4.1) were cultured and inoculum prepared for phenotypic screening of the F_6 advanced populations. The choice of these four races was based on their robustness in growth and sporulation and their ability to show differences in infection on the susceptible and resistant controls.

To confirm sporulation, *Colletotrichum lindemuthianum* samples were prepared on slides and viewed under X10 and X40 magnification of a compound microscope.

Table 4.4: Grouping of the F_6 advanced lines based on number and type of genes inherited

Group	Number of genes inherited	Genes inherited
Group 1	3	*Co-4^2+Co-5+Co-9*
Group 2	2	*Co-4^2+Co-5*
Group 3	2	*Co-4^2+Co-9*
Group 4	2	*Co-4^3+Co-9*
Group 5	2	*Co-5+Co9*
Group 6	1	*Co-5*
Group 7	1	*Co-4^2*
Group 8	1	*Co-4^3*
Group 9	1	*Co-9*
Group 10	0	No gene inherited

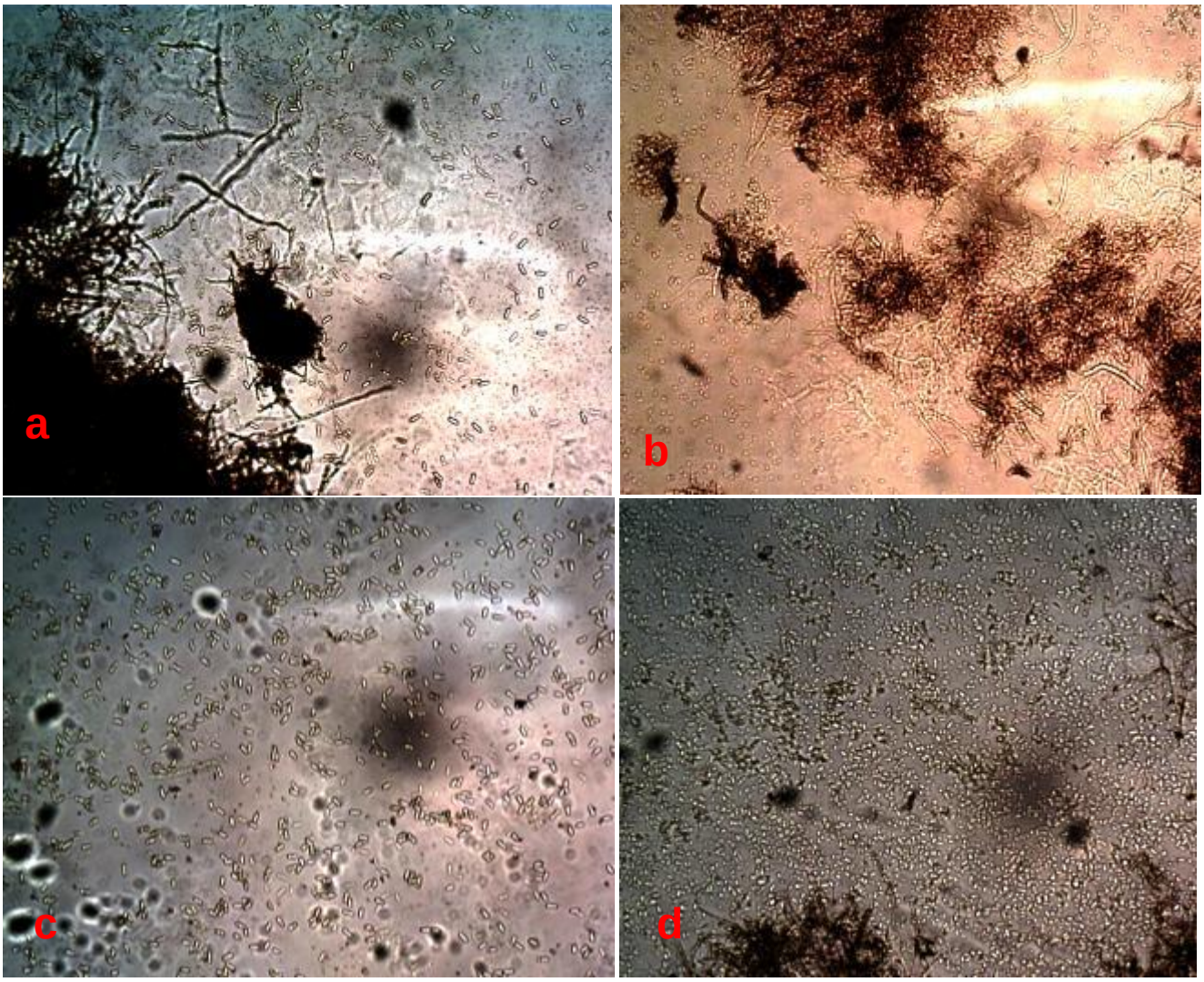

Plate 4.1: Conidia of *C. lindemuthianum* in culture; **a** = Race 352; **b** = Race 713; **c** = Race 767; **d** = Race 2047

Photograghs of *C. lindemuthianum* in sporulation were taken using Optikam Pro 3 microscope digital camera at X40 magnification. The detached leaf technique (Tu, 1986) for screening beans in vitro against anthracnose was used to differentiate the resistant and susceptible bean families.

Leaf trifoliates were detached starting from 14 days after planting and inoculated by immersion in the suspension containing *C. lindemuthianum* spores at a concentration of 1.2 x 10^6 ml^{-1} adjusted using a hemocytometer and a compound microscope. The inoculated leaf trifoliates were placed in transparent plastic containers with moistened paper towels and covered with transparent covers, which were tightly placed to allow for humidity build-up. The containers were placed on wooden shelves fitted with Phillips^R TLT 18-20W/75RS Fluorescents tubes that supplied approximately 50µmoles $m^{-2}s^{-1}$ of light so as to enable prolonged physiological processes of the detached leaves up to between 14 to 28 days.

A light timer was connected to the fluorescent tubes to enable 12-hour day light and 12-hour night regime. Room temperatures were maintained, with the help of an air conditioner, between 22^{o}C and 25^{o}C, which is recommended for successful infection of *C. lindemuthianum* on the bean host (Awale *et al.*, 2007).

Disease symptoms were scored after a seven day incubation period using a modified 1 – 9 scale by Balardin et al. (1997) where; 1 = no symptoms (resistant), 2 – 3 = very small lesions mostly on primary leaves (resistant), 4 – 9 = numerous enlarged lesions or sunken cankers on the lower side of the leaves or hypocotyls (susceptible); and plants were categorized as either Resistant (R) or Susceptible (S).

4.2.5 Data analysis

Disease severity data was subjected to Analysis of Variance (ANOVA) using GenStat Discovery, 12th Edition (Anonymous, 2009). To determine whether pyramid group means were significantly different with respect to anthracnose resistance levels, a Tukey's Honest Significant Difference (HSD) test was carried out to test the null hypothesis H_o;

all gene pyramid group means are equal; $H_o : \mu_i = \mu_j$, $H_a : \mu_i \neq \mu_j$. Tukey Test statistic; $HSD = q\sqrt{MSE/n_c}$. Where; q = value from studentized range table, MSE = Mean Square for Error from ANOVA table, n_c = number of replicates per treatment. Standard error of pyramid group means (SEM) was computed using the formula; $SEM = s/\sqrt{n}$, where; s = sample standard deviation and n = sample size. Sample standard deviation (s) was computed using the formula; $s = \sqrt{1/N-1} \sum^{N}_{i=1}(x_i - x)^2$, where; x_1 …., x_N = the sample data set, x = mean value of the data set, N = size of sample data set.

4.3 Results

4.3.1 F_6 populations with pyramided and single resistance genes

Advanced common bean families at F_6 generation, their pedigrees and molecular marker and resistance genes inherited are presented in Table 4.5.

Table 4.5: Sixty nine (69) F_6 families with their profile of inherited resistance genes

		Markers and Genes screened				
		SAS13	***SBB14***	***SAB3***	***SB12***	***PYAA19$_{800}$***
Pedigree[a]	**Family codes**	***Co-4^2/ Co-4^3***	***Co-4^2***	***Co-5***	***Co-9***	***Prr***
12x8xRWR719xK132	44.5.7.1.1.1.1	-	-	-	+	-
12x8xRWR719xK132	44.5.7.1.1.1.4	-	-	-	+	-
12x8xRWR719xK132	44.5.7.1.1.1.7	-	-	-	+	-
12x8xRWR719xK132	16.1.2.1.6.3.1	-	-	-	-	-
12x8xRWR719xK132	16.1.2.1.6.3.2	-	-	-	-	-
12x8xRWR719xK132	16.1.2.1.6.3.3	-	-	-	-	-
12x8xRWR719xK132	16.1.2.1.6.3.5	-	-	-	-	-
12x8xRWR719xK132	16.1.2.3.7.1.1	-	-	-	-	+
12x8xRWR719xK132	16.1.2.3.7.1.3	-	-	-	-	+
12x8xRWR719xK132	16.1.2.3.7.1.4	-	-	-	-	+
12x8xRWR719xK132	16.6.1.6.25.1.2	+	+	-	-	-
12x8xRWR719xK132	44.5.2.1.26.1.4	+	+	+	-	-
12x8xRWR719xK132	44.5.2.3.28.1.1	+	+	+	+	-
12x8xRWR719xK132	44.5.2.3.28.1.2	+	+	+	+	-
12x8xRWR719xK132	44.5.2.3.28.4.3	+	+	+	+	-
12x8xRWR719xK132	44.5.2.3.28.4.5	+	+	+	+	-
12x8xRWR719xK132	44.5.2.3.28.4.6	+	+	+	+	-
12x8xRWR719xK132	44.5.2.3.28.4.8	+	+	+	+	-
12x8xRWR719xNABE4	44.7.9.8.102.1.1	+	+	+	+	-
12x8xRWR719xNABE4	44.7.5.8.112.1.2	-	-	-	-	-
12x8xRWR719xNABE4	44.7.5.8.112.1.3	-	-	-	-	-
12x8xRWR719xNABE4	44.7.5.8.112.1.4	-	-	-	-	-

12x8xRWR719xNABE4	44.7.5.8.112.2.1	-	-	-	-	-
12x8xRWR719xNABE4	89.5.2.7.117.1.1	+	+	-	+	-
12x8xRWR719xNABE4	89.5.2.10.118.15.2	+	-	-	+	-
12x8xRWR719xNABE4	44.7.2.2.76.1.1	+	+	+	+	+
12x8xRWR719xNABE4	44.7.2.2.76.4.1	+	+	+	+	+
12x8xRWR719xNABE4	89.5.1.1.81.1.1	+	+	-	-	+
12x8xRWR719xNABE4	89.5.1.1.81.2.1	+	+	-	-	+
12x8xRWR719xNABE4	44.7.8.2.91.10.1	+	+	-	-	-
12x8xRWR719xNABE4	44.7.8.2.91.10.3	+	+	-	-	-
12x8xRWR719xNABE4	44.7.8.2.91.10.4	+	+	-	-	-
12x8xRWR719xNABE4	44.7.8.2.91.11.2	+	+	-	-	-
12x8xRWR719xNABE4	44.7.8.2.91.11.3	+	+	-	-	-
12x8xRWR719xNABE4	44.7.9.5.92.2.3.4.6	+	+	-	-	-
12x8xRWR719xNABE4	44.7.8.3.92.3.2	+	+	-	-	-
12x8xRWR719xNABE4	44.7.8.3.92.3.5	+	+	-	-	-
12x8xRWR719xNABE13	44.1.6.7.130.1.1	-	-	-	-	-
12x8xRWR719xNABE13	44.1.6.7.130.3.2	-	-	-	-	-
12x8xRWR719xNABE13	16.1.3.8.136.1.4	+	-	-	-	+
12x8xRWR719xNABE13	16.1.3.8.136.2.5	+	-	-	-	+
12x8xRWR719xNABE13	16.1.3.8.136.2.7	+	-	-	-	+
12x8xRWR719xNABE13	16.1.3.8.136.2.8	+	-	-	-	+
12x8xRWR719xNABE13	16.1.3.8.136.2.9	+	-	-	-	+
12x8xRWR719xNABE13	44.1.4.3.141.1.2	-	-	+	+	+
12x8xRWR719xNABE13	44.1.4.3.141.1.3	-	-	+	+	+
12x8xRWR719xNABE13	44.1.4.3.141.1.4	-	-	+	+	+
12x8xRWR719xNABE13	44.1.4.3.141.2.1	-	-	+	+	+
12x8xRWR719xNABE13	44.1.4.5.142.3.6	-	-	+	-	+
12x8xRWR719xNABE13	44.1.4.5.142.4.2	-	-	+	-	+
12x8xRWR719xNABE13	44.1.4.5.142.4.4	-	-	+	-	+
12x8xRWR719xNABE13	44.1.4.5.142.4.6	-	-	+	-	+
12x8xRWR719xNABE13	44.1.4.5.142.4.7	-	-	+	-	+
12x8xRWR719xNABE13	44.1.4.7.143.1.4	-	-	+	-	-
12x8xRWR719xNABE13	44.1.4.7.143.1.8	-	-	+	-	-
12x8xRWR719xNABE14	16.3.3.1.151.1.1	-	-	+	-	+
12x8xRWR719xNABE14	16.3.3.1.151.1.2	-	-	+	-	+
12x8xRWR719xNABE14	16.3.3.2.152.1.2.1	-	-	-	-	-
12x8xRWR719xNABE14	16.3.3.2.152.1.2.2	-	-	-	-	-
12x8xRWR719xNABE14	16.3.3.8.157.3.3	+	+	+	-	+
12x8xRWR719xNABE14	16.3.3.9.158.1.2	+	-	+	-	+
12x8xRWR719xNABE14	16.3.3.9.158.1.3	+	-	+	-	+
12x8xRWR719xNABE14	16.3.3.10.159.6.2	+	+	+	-	+
12x8xRWR719xNABE14	16.3.3.11.160.2.1	+	+	+	-	+
12x8xRWR719xNABE14	16.3.3.11.160.2.2	+	+	+	-	+
12x8xRWR719xNABE14	16.3.3.11.160.2.3	+	+	+	-	+
12x8xRWR719xNABE14	16.3.3.11.161.1	-	-	+	+	+
12x8xRWR719xNABE14	16.3.3.11.161.3	-	-	+	+	+
12x8xRWR719xNABE14	16.3.3.11.161.4	-	-	+	+	+

[a] 12 = Donor parent G2333; 8 = Donor parent PI 207262; + = presence of band on gel; - = absence of band on gel.

A total of 53 $F_{4:5}$ and 69 $F_{4:6}$ families with varying resistance gene profiles were obtained after the pedigree and fixation steps of the cascading gene pyramiding scheme (Servin, *et al*., 2004). Out of 69 $F_{4:6}$ families, nine (9) families inherited all the three (3) target anthracnose genes (*Co-4*^{2}/*Co-4*^{3}, *Co-5* and *Co-9*), 17 families inherited two anthracnose resistance genes, 28 families inherited a single one anthracnose resistance gene; while 12 families did not inherit any of the target genes (Table 4.5). Twenty seven (27) families possessed *Co-4*^{2} allele, eight (8) families possessed *Co-4*^{3} allele, 33 families possessed *Co-5* gene, while 21 families possessed *Co-9* gene (Table 4.5).

4.3.2 Specific molecular marker analysis and polymorphism

Gel photographs of the four dominant SCAR markers *SAS13, SAB3, SB12, PYAA19*$_{800}$ and one codominant marker *SBB14* are presented on Plate 4.2.

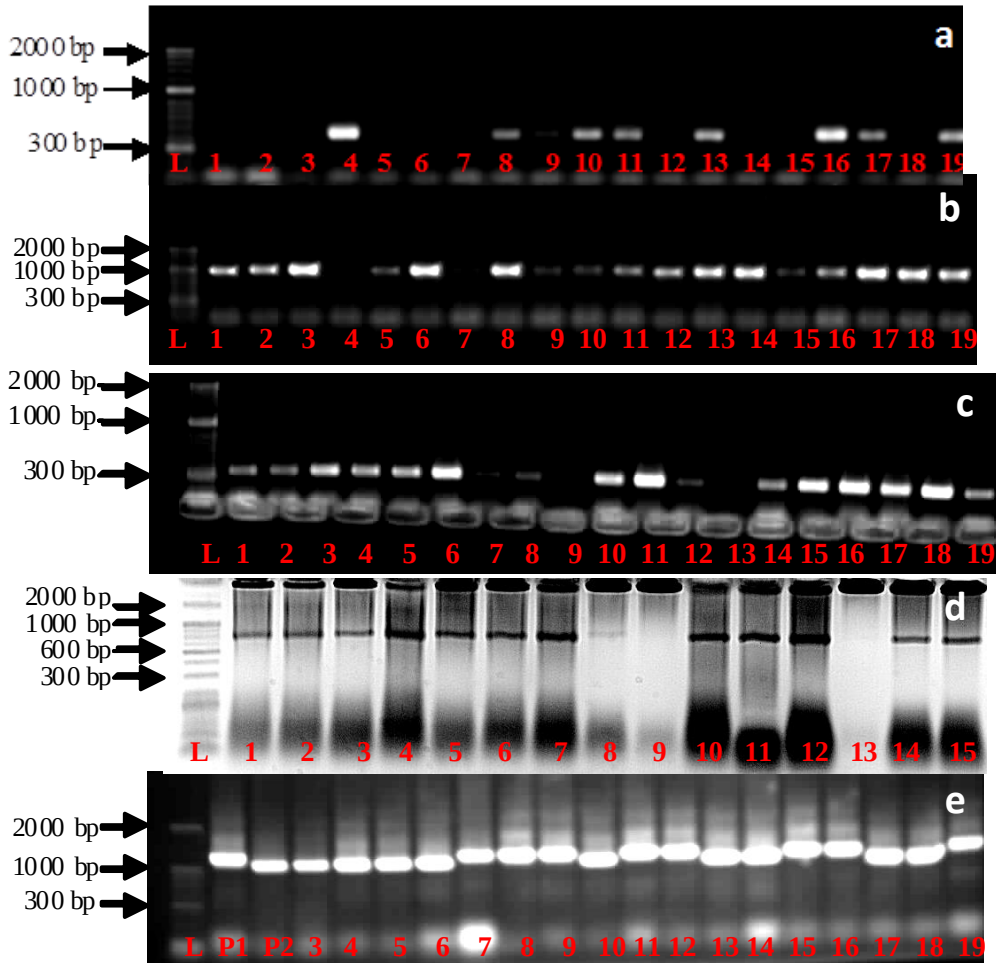

Plate 4.2: Gel photos showing banding patterns of DNA fragments amplified with the different SCAR markers.
a = DNA fragment at 400bps amplified with *SAB3* primer linked to *Co-5* gene; **b** = DNA fragment at 950bps amplified with *SAS13* primer linked to *Co-4* locus; **c** = DNA fragment at 350bps amplified with *SB12* primer linked to *Co-9* gene; **d** = DNA fragment at 800bps amplified with *KL1KR1* primer of the *PYAA19*$_{800}$ marker
e = Two DNA fragments at 1150bps for upper fragment and 1050bps for lower fragment amplified with *SBB14* codominant primer linked to allele *Co-4*^{2}.
Lanes: *L* 2000bps molecular weight marker ladder; *1 – 19* segregating lines; *P1* Donor parent; *P2* Susceptible parent

The markers were polymorphic between the resistant and susceptible parents and were successfully used to distinguish genotypes where the target gene was present from those where the gene was absent (Plate 4.2). A total of 1,704 PCR reactions were performed with the five markers in four MAS cycles out of which 893 were positive reactions. SAS13 was the most frequent marker with 64.2% of 372 reactions followed by SB12 (58.6% of 372 reactions), SBB14 (47.0% of 317 reactions), $PYAA19_{800}$ (45.4% of 271 reactions) and SAB3 (44.1% of 372 reactions).

4.3.3 Evaluation of families for resistance to anthracnose

The reaction of 36 advanced F_4:$_6$ families and seven (7) parent cultivars to four *C. lindemuthianum* races and four SCAR markers is presented in Table 4.6.

Table 4.6: Reaction of pyramided lines and parents to four races of *C. lindemuthianum* and four SCAR markers

Family code	Phenotypic data				Genotypic data			
	C. lindemuthianum races				Molecular markers			
	2047	713	767	352	SAS13	SAB3	SB12	$PYAA19_{800}$
44.5.7.1.1.1.1	S	S	S	R	0	0	1	0
16.1.2.1.6.3.3	S	S	S	S	0	0	0	0
16.1.2.1.6.3.5	S	S	S	S	0	0	0	0
16.1.2.3.7.1.4	S	S	S	S	0	0	0	1
16.6.1.6.25.1.2	S	R	R	R	1	0	0	0
44.5.2.1.26.1.4	S	S	S	S	0	0	0	0
44.5.2.3.28.1.1	S	R	S	R	0	1	1	0
44.5.2.3.28.1.2	S	R	S	R	0	1	1	0
44.5.2.3.28.4.5	R	R	R	R	1	1	1	0
44.5.2.3.28.4.8	R	R	R	R	1	1	1	0
44.7.2.2.76.1.1	R	R	R	R	1	1	1	1
44.7.2.2.76.4.1	R	R	R	R	1	1	1	1
89.5.1.1.81.1.1	R	R	R	R	1	0	0	1
44.7.8.2.91.10.3	R	R	R	R	1	0	0	0
44.7.8.2.91.10.4	R	R	R	R	1	0	0	0
44.7.9.5.92.2.3.4.6	R	R	R	R	1	0	0	0
44.7.9.8.102.1.1	R	R	R	R	1	1	1	0
44.7.5.8.112.1.3	S	S	S	S	0	0	0	0
89.5.2.7.117.1.1	S	R	R	R	1	0	1	0
89.5.2.10.118.15.3	S	S	S	R	1*	0	1	0
44.1.6.7.130.1.1	S	S	S	S	0	0	0	0
16.1.3.8.136.1.4	R	R	S	R	1*	0	0	1
16.1.3.8.136.2.7	S	R	S	R	1*	0	0	1

16.1.3.8.136.2.8	S	R	S	R	1*	0	0	1
44.1.4.3.141.1.2	R	R	R	R	0	1	1	1
44.1.4.3.141.1.4	R	R	R	R	0	1	1	1
44.1.4.5.142.4.2	R	R	R	R	0	1	0	1
44.1.4.5.143.1.4	R	R	R	R	0	1	0	0
16.3.3.1.151.1.1	S	S	S	S	0	1	0	1
16.3.3.1.151.1.2	R	R	R	R	0	1	0	1
16.3.3.2.152.1.2.2	S	S	S	S	0	0	0	0
16.3.3.9.158.1.2	R	R	R	R	1	1	0	1
16.3.3.9.158.1.3	R	R	R	R	1	1	0	1
16.3.3.11.160.2.2	S	R	R	R	1	1	0	1
16.3.3.11.160.2.3	R	R	R	R	1	1	0	1
42.12.4.1.161.4	S	S	S	R	0	0	1	1
Parents								
PI207262	S	S	S	R	1*	0	1	0
Nabe 4	S	S	S	S	0	0	0	0
RWR719	S	S	S	S	0	0	0	1
G2333	R	R	R	R	1	1	0	0
K132	S	S	S	S	0	0	0	0
Nabe 13	S	S	S	S	0	0	0	1
Nabe 14	S	S	S	S	0	0	0	1

* *Co-4*^{3} allele was identified in segregating populations by simultaneously running SBB14 and SAS13 markers on the samples

[a] 12 is the position number of cultivar G2333 while 8 is position number of cultivar PI207262 in the anthracnose differential series

R = resistant reaction, S = Susceptible reaction; 1 = marker present, 0 = marker absent

SAS13 marker tags *Co-4* locus (both *Co-4*^{2} & *Co-4*^{3} alleles), SAB 3 marker tags *Co-5* gene, SB12 marker tags *Co-9* gene and $PYAA19_{800}$ marker tags *Pythium* resistance gene

The five (5) families that possessed the three (3) anthracnose pyramided genes *Co-4*^{2}, *Co-5* and *Co-9* were resistant to all the four races. Three (3) of the four (4) families possessing *Co-4*^{2} and *Co-5* genes were also resistant to all four (4) races, while two families with the same pyramid succumbed to the aggressive races 2047 and 767. Four (4) out of five (5) families possessing single gene *Co-4*^{2} were resistant to all the four races, while families with only *Co-4*^{3} allele succumbed to races 2047 and 767. Four of the five families possessing single gene *Co-4*^{2} were resistant to all the four races, while families that possessed the allele *Co-4*^{3} succumbed to races 2047 and 767. The two families that possessed single gene *Co-9* and the family that possessed *Co-4*^{3}*+Co-9* pyramid succumbed to three aggressive races 2047, 713 and 767 and showed resistance to only race 352. The donor parents PI207262 possessing the *Co-4*^{3}*+Co-9* pyramid was only resistant to race 352, while the donor parent G2333 possessing *Co-4*^{2}*+Co-5+Co-7* pyramid was resistant to all four races. All six families that did not inherit any resistance

gene for anthracnose were susceptible to all four races. Three of four families possessing *Co-5* single gene were resistant to all the four races. Reactions to *C. lindemuthianum* of leaf trifoliates of F_6 and parental cultivars are presented in Plates 4.3A and 4.3B. Presence of the *Co-9* gene was always associated with symptoms except in the three-gene pyramid (Plate 4.3A) and Single genes showed effectiveness resistance against *C. lindemuthianum* (Plate 4.3B).

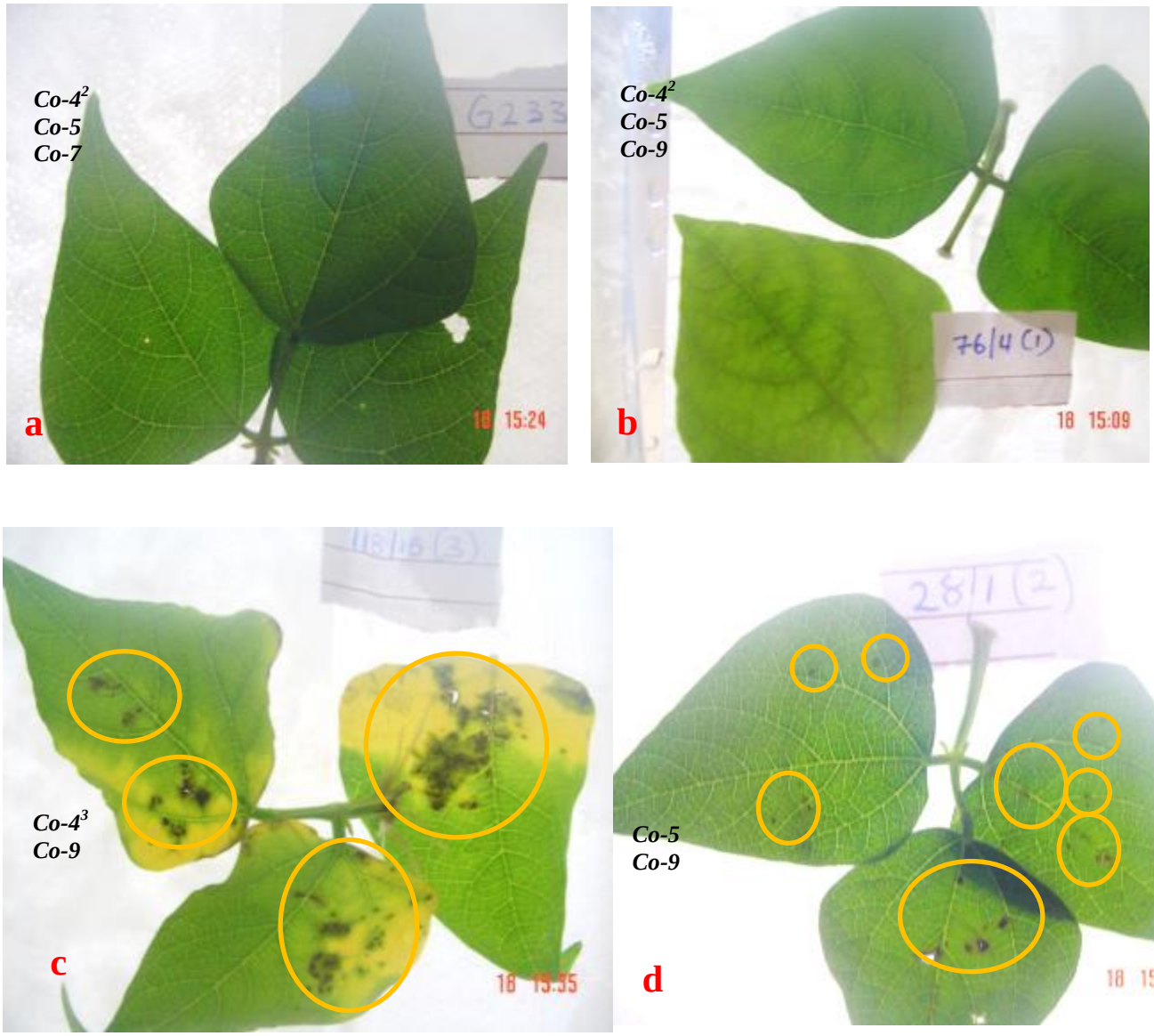

Plate 4.3A: Leaf trifoliates of three F6 cultivars with pyramided resistance genes in comparison with a known resistant cultivar seven days post inoculation; **a** = resistance donor parent G2333 with no symptoms; **b** = cultivar with a three-gene pyramid (*Co-4²+Co-5+Co-9*) showing no symptoms; **c** = cultivar with a two-gene pyramid (*Co-4³+Co-9*) showing severe symptoms; **d** = cultivar with a two-gene pyramid (*Co-5+Co-9*) showing mild symptoms

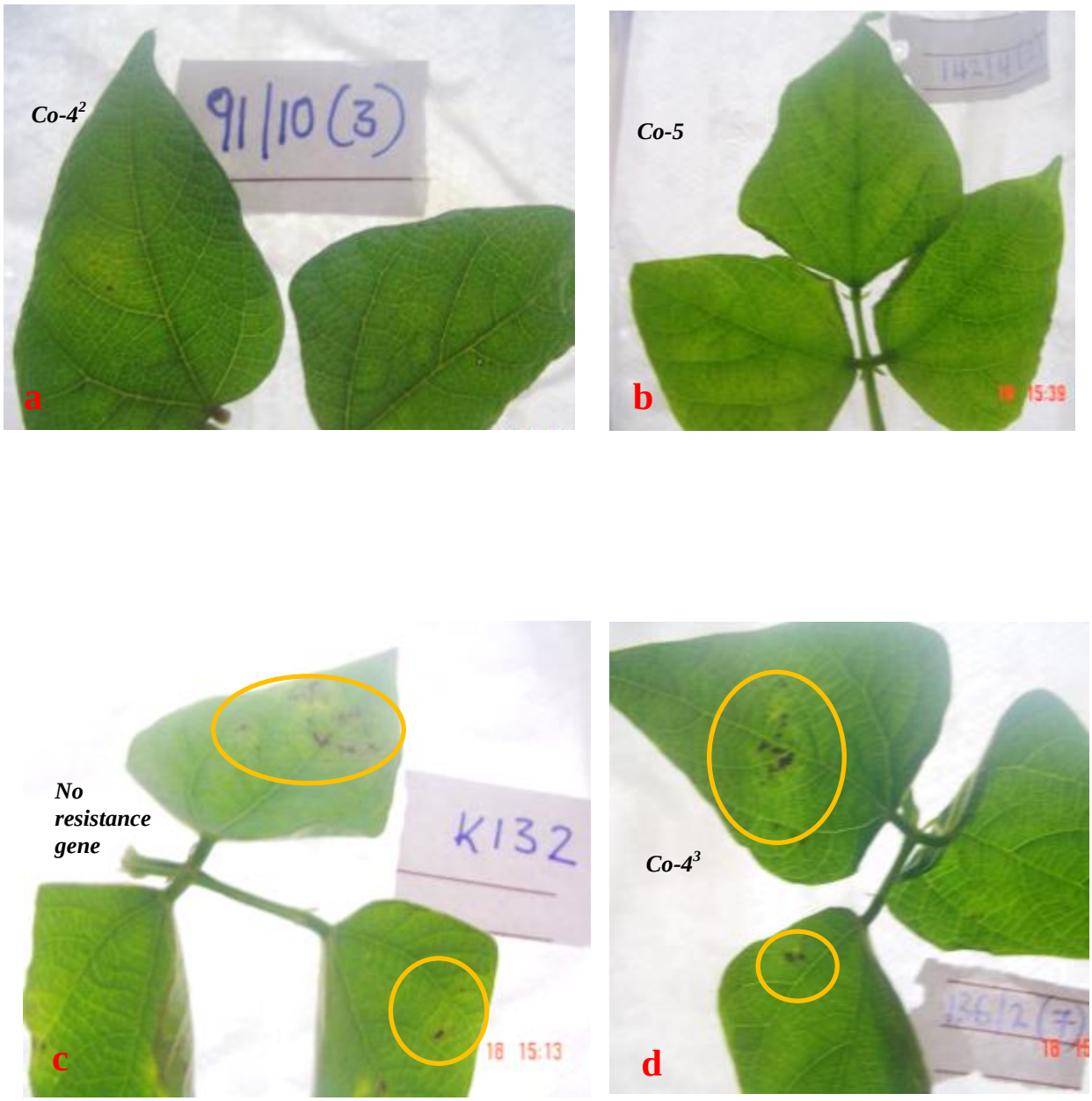

Plate 4.3B: Leaf trifoliates of three F_6 cultivars with single resistance genes in comparison with one susceptible cultivar, seven days post inoculation; **a** = F_6 cultivar with *Co-4*^{2} single gene showing no symptoms; **b** = F_6 cultivar with Co-5 single gene showing no symptoms; **c** = Susceptible commercial cultivar showing symptoms; **d** = F_6 cultivar with *Co-4*^{3} single gene showing mild symptoms;

4.3.4 Effectiveness of single and pyramided genes in conferring broad resistance to diverse *C. lindemuthianum* races

Analysis of variance for anthracnose disease severity is presented in Table 4.7.

Table 4.7: Analysis of variance for severity of four races on different Gene-groups

Source of variation	d.f.	S.S	M.S	%Contribution	F pr.
Rep	2	0.175	0.087	0.15	
Gene-groups	9	276.121	30.680*	52.55	<.001
Race	3	76.547	25.516*	43.70	<.001
Gene-group x Race	27	53.581	1.985*	3.40	<.001
Residual	78	9.060	0.116	0.20	

Gene-groups, races and interaction between Gene-groups and races were all highly significant ($p < 0.01$) with Gene-groups contributing highest (52.6%) to the total variation followed by Races (43.7%) and Gene-group by Race interaction (1.98%). Severity scores of cultivars in the different gene groups inoculated with diverse *C lindemuthianum* races are presented in Table 4.8.

Table 4.8: Mean severity scores of cultivars in the different gene groups inoculated with diverse *C. lindemuthianum* races

		Races				
Group	***Pyramid groups***	**352**	**713**	**767**	**2047**	**Overall Group mean**
1	*Co-4^2+Co-5+Co-9*	1.0±0.00	1.1±0.07	1.1±0.07	1.1±0.07	**1.1±0.03**
2	*Co-4^2+Co-5*	1.1±0.13	2.2±0.12	1.9±0.18	3.1±0.24	**2.1±0.41**
3	*Co-4^2+Co-9*	1.0±0.00	2.0±0.00	2.0±0.00	4.3±0.33	**2.3±0.69**
4	*Co-4^3+Co-9*	1.5±0.29	5.8±0.17	6.3±0.44	7.0±0.29	**5.2±1.24**
5	*Co-5+Co-9*	1.4±0.07	2.3±0.07	3.3±0.29	4.3±0.29	**2.8±0.63**
Mean		**1.2±0.10**	**2.7±0.81**	**2.9±0.92**	**4.00±0.96**	**2.7±0.68**
	Singe-gene groups					
6	*Co-5*	2.0±0.00	2.5±0.23	2.8±0.07	3.0±0.27	**2.6±0.22**
7	*Co-4^2*	1.0±0.00	1.4±0.12	1.2±0.12	2.4±0.12	**1.5±0.31**
8	*Co-4^3*	1.9±0.10	3.3±0.49	4.3±0.38	4.2±0.11	**3.4±0.56**
9	*Co-9*	2.3±0.17	5.2±0.17	5.5±0.00	4.8±0.17	**4.5±0.73**
Mean		**1.8±0.28**	**3.1±0.80**	**3.5±0.93**	**3.6±0.55**	**3.0±0.63**
10	***No-gene group***	5.4±0.07	5.9±0.07	6.3±0.12	6.3±0.09	**5.9±0.21**
Overall Mean for Races		**1.9±0.42**	**3.2±0.57**	**3.5±0.64**	**4.1±0.56**	**3.1±0.50**

Lsd (0.05) = 0.55, S.e.d = 0.26, C.V = 7.8%

Out of the four *C. lindemuthianum* races, race 2047 had the highest overall mean score (4.1±0.56) followed by races 767 (3.5±0.64), 713 (3.2±0.57) and 352 (1.9±0.42). The *no-gene* group had the highest mean score (5.9±0.21) across the four races, followed by the single-gene groups (3.0±0.63), while the pyramid gene groups had the lowest mean score (2.7±0.68) across the four races. However, the mean scores 3.0±0.63 and 2.7±0.68 for pyramid and single -gene groups respectively were not significantly different from each other (*Lsd 0.05*).

There were five pyramid gene groups with two and three resistance genes which were inoculated with the four races of *C. lindemuthianum* (Table 4.8). The three-gene pyramid group *Co-4*^{2}*+Co-5+Co-9* had the lowest severity score of all pyramid groups. This was evident in the amount of leaf symptoms expressed on leaves of plants in two-gene pyramid groups, such as *Co-4*^{2}*+Co-5* and "*Co-5+Co-9*", in comparison with those with the three-gene pyramid (Plate 4.3). Among the two-gene pyramid groups, the *Co-4*^{3}*+Co-9* pyramid group had the highest overall mean severity score (5.2±1.24) (Plate 4.3A) across the four races and it succumbed to all races except race 352 which was the least aggressive. It was followed by *Co-5+Co-9* (2.8±0.63) and *Co-4*^{2}*+Co-9* (2.3±0.69), both of which succumbed to race 2047. The pyramid group *Co-4*^{2}*+Co-5* had the lowest severity score and did not succumb to any race (≥3.1).

Pair-wise comparisons of group means are presented in Table 4.9. The mean severity score 1.1±0.03 for the three-gene pyramid *Co-4*^{2}*+ Co-5+Co-9* was significantly lower (*P<0.01*) than all the scores of the two-gene pyramid groups. The mean score 2.1±0.41 for *Co-4*^{2}*+Co-5* pyramid group was significantly lower (*P<0.05, P<0.01*) than scores 2.8±0.63 and 5.2±1.24 for the *Co-5+Co-9* and *Co-4*^{3}*+Co-9* pyramid groups respectively, but was not significantly different from score 2.3±0.69 for *Co-4*^{2}*+Co-9* pyramid group.

The mean scores 2.8±0.63 (*Co-5+Co-9*) and 5.2±1.24 (*Co-4*^{3}*+Co-9*) were significantly different from each other. The mean score 2.3±0.69 for the *Co-4*^{2}*+Co-9* pyramid group was significantly lower than score 5.2±1.24 (*Co-4*^{3}*+Co-9*) but was not significantly different (*P<0.01*) from 2.8±0.63 (*Co-5+Co-9*).

Table 4.9: Pair-wise comparison of mean severity scores of the different gene groups

Gene group		*Co-4^2+ Co-5 + Co-9*	*Co-4^2+Co-5*	*Co-4^2+Co-9*	*Co-4^3+Co-9*	*Co-5+Co-9*	*Co-5*	*Co-4^2*	*Co-4^3*	*Co-9*	*No-gene*
	Means	**1.1**	**2.1**	**2.3**	**5.2**	**2.8**	**2.6**	**1.5**	**3.4**	**4.5**	**5.9**
Co-4^2+ Co-5+Co-9	**1.1**										
Co-4^2+Co-5	**2.1**	P<0.01									
Co-4^2+Co-9	**2.3**	P<0.01	n/s								
Co-4^3+Co-9	**5.2**	P<0.01	P<0.01	P<0.01							
Co-5+Co-9	**2.8**	P<0.01	P<0.05	n/s	P<0.01						
Co-5	**2.6**	P<0.01	n/s	n/s	P<0.01	n/s					
Co-4^2	**1.5**	n/s	n/s	P<0.01	P<0.01	P<0.01	P<0.01				
Co-4^3	**3.4**	P<0.01	P<0.01	P<0.01	P<0.01	n/s	P<0.01	P<0.01			
Co-9	**4.5**	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01		
No-gene	**5.9**	P<0.01	P<0.01	P<0.01	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	

Critical values $HSD_{0.05}$ = 0.63, $HSD_{0.01}$ = 0.78, n/s = not significant

There were four single-gene groups namely *Co-4^2*, *Co-4^3*, *Co-5*, *Co-9* and one no-gene group. The *Co-4^2* gene group had the lowest severity score (1.5±0.31) followed by *Co-5* (2.6±0.22), *Co-4^3* (3.4±0.56) and *Co-9* (4.5±0.73) gene groups. The no-gene group had the highest severity score (5.9±0.21). Overall severity scores of single-gene groups were significantly different (*P<0.01*) from each other and from the *no-gene* group. The mean severity score of *Co-9* gene group (4.5±0.73) was significantly higher than mean scores of all the single-gene groups and was the only group with severity score falling in the susceptible range (4.0 – 9.0).

The mean severity score of the pyramid groups was 2.7±0.68 while the mean severity score of the single-gene groups was 3.0±0.63 (Table 4.8). These two means were not significantly different from each other although both were significantly different from the 5.9±0.21 mean score of the *No-gene* group (*P*<0.01, $HSD_{0.05}$ = 0.58, $HSD_{0.01}$ = 0.72). Severity scores of no-gene, single-gene and pyramid gene groups inoculated with four *C. lindemuthianum* races are presented graphically in Figure 4.1.

The mean score, 1.5±0.31, of the *Co-4^2* single-gene group was not significantly different from the mean scores, 1.1±0.03 and 2.1±0.41, of the *Co-4^2+Co-5+Co-9* and *Co-4^2+Co-5*

pyramid groups respectively, but was significantly lower than the mean scores of the *Co-4*^{2}*+Co-9* (2.3+0.69), *Co-4*^{3}*+Co-5* (5.2±1.24) and *Co-5+Co-9* (2.8±0.63) pyramid groups.

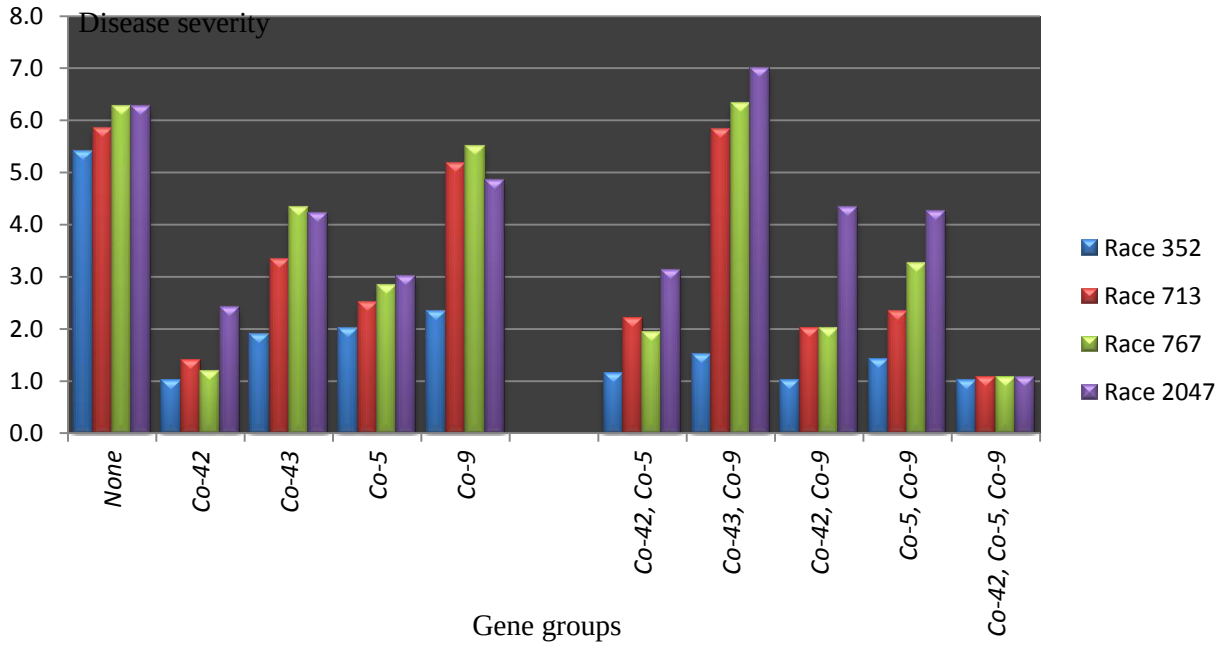

Figure 4.1: Severity levels of the different single-gene and pyramid-gene groups screened with four races of *C. lindemuthianum*

The mean score, 2.6±0.22, of the *Co-5* single-gene group was not significantly different from mean scores 2.1±0.41, 2.3±0.69 and 2.8±0.63 of the *Co-4*^{2}*+Co-5*, *Co-4*^{2}*+Co-9* and *Co-5+Co-9* pyramid groups respectively, but was significantly higher than the score 1.1±0.03 for the three-gene pyramid *Co-4*^{2}*+Co-5+Co-9* ($P<0.01$, $HSD_{0.05} = 0.63$, $HSD_{0.01} = 0.78$).

The mean score 3.4±0.56 of the *Co-4*^{3} single-gene group was not significantly different from 2.8±0.63 for *Co-5+Co-9* the pyramid but was significantly less than 5.2±1.24 for the *Co-4*^{3}*+Co-9* pyramid group. The mean score 4.5±0.73 of the *Co-9* gene group was significantly higher than scores for all the pyramid gene groups.

4.4 Discussion

4.4.1 Gene pyramiding and development of advanced populations

Developing elite breeding populations and varieties requires plant breeders to combine desirable traits from multiple parents in gene pyramiding, which can be accelerated by the

use of molecular markers. In this study four parents (K132, NABE4 NABE13 & NABE14) susceptible to anthracnose were crossed with G2333, PI207262 and RWR719 in a three cross gene pyramid scheme. The SCAR markers *SAS13*, *SH18, SBB14*, *SAB3* and *SB13* were used for selection of anthracnose resistance genes. The inclusion of parent RWR719 in the breeding scheme and MAS screening using the SCAR marker $PYAA19_{800}$ were done to introduce Pythium root rot resistance gene especially among populations that had K132 and NABE 4 susceptible parents, which had an added advantage of background improvement for root rot resistance among the populations.

Data for SCAR marker SB12 revealed the presence of the *Co-9* anthracnose resistance gene in the parent RWR719. The efficiency of a gene pyramiding program can be improved through strategies that enable recovery of the ideotype in the shortest duration and in the cheapest way. Servin et al. (2004) recommended crossing the root genotype with a blank parent to obtain a genotype carrying all favorable alleles in coupling phase then selfing the F_1 to produce the ideotype with fixed target genes in one generation.

4.4.2 Marker analysis and polymorphism during gene pyramiding

It was observed that frequency of appearance of a marker in a population increased as the linkage distance of the marker from the target gene decreased. Among the four markers, *SAB3* was reported by Campa et al. (2005) to be the most distant marker from the gene of interest at 14.4cM, followed by SBB14 linked to the $Co\text{-}4^2$ allele at 5.89cM (Awale and Kelly, 2001). SB12 was reported to be linked to the *Co-9* gene at a distance of 2.9cM (Mendez de Vigo *et al*., 2002) while SAS13 was reported to be the most tightly linked to the *Co-4* locus at 0.01cM (Young *et al*., 1998). According to Byrne and Richardson (2005), 1cM of genetic distance is approximately equal to 1% recombination and hence, as the distance between the marker and the gene of interest increases, there is a greater chance of recombination between gene and marker. This implies that, in this study, the chances of recombination leading to false positives were highest with SAB3 and least with SAS13.

The breeder's capacity to identify the desired genotype is one of the factors that determine the success of gene pyramiding. The use of tightly linked markers has greatly enhanced this capacity. The two donor parents G2333 and PI207262 have different alleles at the same locus *Co-4* namely $Co\text{-}4^2$ and $Co\text{-}4^3$. The two alleles were both detected by the SAS13 marker without differentiation because SAS13 marker amplifies consensus sequences common to all alleles at the *Co-4* locus. The co-dominant marker SBB14 specifically amplified the $Co\text{-}4^2$

allele which agrees with the findings of Awale and Kelly (2001), while no marker was available to specifically amplify the *Co-4*^{3} allele. This challenge was overcome by running both SAS13 and SBB14 markers on the same samples, which made it possible to differentiate between genotypes possessing *Co-4*^{2} allele from those possessing *Co-4*^{3} allele.

4.4.3 Effectiveness of single and pyramided genes in conferring broad resistance to bean anthracnose

Results for analysis of variance revealed high significance among Gene-groups (genotypes), Races and 'Gene-group x Race' interactions ($P<0.001$). This implies that the number of genes/ gene combinations and *C. lindemuthianum* races significantly affected disease severity and are therefore of relative importance in the development and progress of the bean anthracnose disease. The significance of 'Gene-group x Race' interaction indicates that the effect of *C. lindemuthianum* races on disease severity highly depended on number and combination of resistance genes in the bean cultivars.

The races 2047 and 767 were the most aggressive and caused the highest disease symptoms on cultivars. They both caused high host susceptibility on cultivars possessing the *Co-4*^{3}*+Co-9* and moderate susceptibility on cultivars possessing *Co-4*^{2}*+Co-9* and *Co-5+Co-9* pyramids and *Co-4*^{3} and *Co-9* single genes. This implies that these gene pyramids and single genes do not effectively confer resistance to the two races.

The race 713 caused moderate susceptibility on cultivars possessing *Co-4*^{3}*+Co-9* pyramid and *Co-9* single gene implying that the pyramid combination and the single gene *Co-9* were not effective against it. The race 352 did not cause susceptibility on any cultivar apart from cultivars with no resistance gene suggesting that all the single genes and the different pyramid combinations were effective against it.

The single genes *Co-4*^{2} and *Co-5* conferred resistance to all the four races 352, 713, 767 and 2047. The *Co-4*^{3} gene conferred resistance to races 352 and 713 but was overcome by races 767 and 2047. The *Co-9* group conferred resistance to only race 352 while the *No-gene* group was overcome by all the four races. These results reveal that *Co-4*^{2} gene was the most effective single gene in conferring broad resistance to bean anthracnose, followed by *Co-5* and *Co-4*^{3} genes. However, the *Co-9* gene was the least effective and it was observed that it was associated with increased severity when combined with another gene. This implies that

the *Co-9* gene was antagonistic with other genes and therefore, should be avoided in gene pyramiding programs. Its presence in the three gene pyramid *Co-4^2+Co-5+Co-9*, however, was not antagonistic probably because of the presence and combined effectiveness of the *Co-4^2* and *Co-5* genes reducing symptoms to a mean score of 1.1±0.03 as compared to the score of 2.1±0.41 in *Co-4^2+Co-5* pyramid where *Co-9* was absent. The results suggest that programs with limited time and funding the single gene *Co-4^2* would be the most appropriate for deployment.

Kelly and Vallejo (2004) reported the *Co-9* gene to possess a very specific breeding value against Andean races of *C. lindemuthianum.* Alzate-Marin et al. (2003) reported the *Co-9* gene to be susceptible to weak Mesoamerican races 65 and 69. This could explain the poor resistance spectrum observed with the *Co-9* gene in this study. The cultivar PI 207262 possessing the *Co-9* gene was reported to be overcome by many anthracnose races (Kelly, 2004) implying the ineffectiveness of the *Co-9* gene as a resistance source. Kelly and Vallejo (2004) recommended its use only to diversify resistance in gene pyramids because of its independence and potential value in controlling Andean races.

Results showed that the single gene *Co-4^2* was as effective as the *Co-4^2+Co-5 and Co-4^2+Co-5+Co-9* pyramids and the single gene *Co-5* was as effective *Co-4^2+Co-5* and *Co-4^2+Co-9* pyramids (Table 4.9). This implies that the two single genes possess factors that promote broad-spectrum resistance and that these factors are higher in *Co-4^2* gene. These two genes would confer effective and broad-spectrum resistance in single deployment against a diverse *C. lindemuthianum* population. The *Co-5* gene was reported to be among the most effective genes in Central America and Mexico but with limited use by bean breeders (Kelly and Vallejo, 2004). It was reported to possess a wide resistance spectrum conferring resistance to 31 races (Balardin *et al*, 1997).

The *Co-4^2* gene was reported to exhibit the most broad-based resistance against *C. lindemuthianum* in common beans (Young and Kelly, 1996; Balardin and Kelly, 1998; Awale and Kelly, 2001). It is located at the *Co-4* locus which has control of up to 97% of all currently identified races of *C. lindemuthianum* (Melloto *et al*., 2000). Young et al. (1998) reported the *Co-4* locus to be a complex gene family and three anthracnose resistance alleles residing at this locus namely *Co-4^2* in cultivars G2333 and SEL 1308, *Co-4* allele in cultivar TO (Young et al., 1998) and *Co-4^3* in cultivar PI207262 (Alzarte-Marin *et al*., 2007). In this study, the *Co-4^3* allele showed a mildly susceptible reaction to races 767 and 2047 with mean

scores of 4.3±0.38 and 4.2±0.11 respectively. Its overall mean score of 3.4±0.56 across all four races, however, was still within the resistance range implying that it is still a highly beneficial allele to specific races and has the potential to add value if combined with other compatible single genes in pyramid. The *Co-4*^{3} allele was, however, reported to possess a narrower anthracnose resistance spectrum than the *Co-4* allele in cultivar TO and the *Co-4*^{2} allele in cultivar G2333 (Alzate-Marin *et al*., 2007).

The *Co-4*^{2}*+Co-5+Co-9* and *Co-4*^{2}*+Co-5* pyramid had the lowest severity scores and did not succumb to any of the races implying that the combination and number of genes in these pyramids was highly effective in conferring broad-spectrum resistance to *C. lindemuthianum* in Uganda. The *Co-4*^{2}*+Co-9* and *Co-5+Co-9* pyramid groups conferred resistance to races 352, 713 and 767 (score 1.0-3.3) but were overcome by race 2047 (> 4.0) while the *Co-4*^{3}*+Co-9* pyramid group was overcome by races 713 (5.8±0.17), 767 (6.3±0.44) and 2047 (7.0±0.29) but conferred resistance to only race 352 (1.5±0.29). These results when compared with the mean severity scores of *Co-4*^{2} and *Co-5* further reveal the poor complementarity of the *Co-9* gene.

The results revealed that gene pyramids were not consistent in conferring broad-spectrum resistance to *C. lindemuthianum* races with the exception of the three-gene pyramid *Co-4*^{2}*+Co-5+Co-9* and the two-gene pyramid *Co-4*^{2}*+Co-5* (Figure 4.1). For instance the pyramid *Co-4*^{3}*+Co-9* was overcome by races 713, 767 and 2047 while the pyramids *Co-5+Co-9* and *Co-4*^{2}*+Co-9* were both overcome by the race 2047. This could be the effect of presence of *Co-9* gene in the pyramids and results have indicated that it may have an antagonistic effect in combination with other genes. For this reason, genotypes with *Co-9* gene should be avoided in breeding programs since the gene will negatively affect resistance. The presence of *Co-4*^{2} gene in a pyramid always reduced disease severity on the leaves. In contrast, the single genes *Co-4*^{2} and *Co-5* were not overcome by any of the four races. This implies that some gene combinations interact negatively and fail to confer effective resistance against a diverse *C. lindemuthianum* race population.

It was observed that a higher number of genes in a pyramid significantly reduced the amount of symptoms on the host (Table 4.8) implying that gene pyramiding has the potential of increasing the potency of resistance. The probability hypothesis (Wheeler and Diachun, 1983) states that "cultivars possessing multiple race-specific resistance genes (pyramided genes) owe their durable resistance to a low probability of the pathogen independently

mutating to virulence at multiple avirulent loci corresponding to the host resistance genes". According to this hypothesis the pyramids *Co-4*^{2}*+Co-5+Co-9* and *Co-4*^{2}*+Co-5*, which were the most effective in conferring resistance, are likely to remain effective over a longer period of time. However, the mechanism by which gene pyramids increase durability is still known and there is no strong evidence for gene number as the dominant mechanism for the durability of pyramids (Mundt, 1991). Therefore, it is crucial that breeders identify and use favorable resistance gene combinations in gene pyramiding programs.

Balardin and Kelly (1998) proposed pyramiding genetically diverse but complementary resistance genes in pyramids and deploying them in different regions as the most practical and realistic approach to provide effective long term anthracnose resistance. Kelly (2004) proposed combination of *Co-4*^{2}, *Co-5* and *Co-6* genes in North America and *Co-1*^{2} and *Co-4*^{2} gene pair for Central America. The *Co-1*^{2} gene confers resistance against Andean races while *Co-4*^{2} confers resistance against Mesoamerican races. Resistance gene pyramids which incorporate at least two unique modes of action are reported to delay the evolution of virulent pathotypes (Roush 1998).

The ability of the single genes *Co-4*^{2}, *Co-5* and *Co-4*^{3} to confer broad-spectrum resistance as effective as pyramided genes, and in some cases better than pyramided genes, could be as a result of factors at their respective loci that promote broad-spectrum and durable resistance (Mundt, 2014); while in some pyramids, the genes interact negatively to promote increased disease severity as was the case in this study when *Co-9* gene was combined with another gene. This implies that, for environments in which the disease occurs at moderate or low severity, a single resistance gene may be adequate for a very long period of time (Mundt 2014). The results suggest that a gene pyramiding program would benefit from first understanding the nature of the individual resistance genes in a given *C. lindemuthianum* population and pyramid only those with broad-spectrum complementarity.

4.5 Conclusions

The SCAR markers were effective in tagging all the target resistance genes in the resistance gene pyramiding program. Resistance gene pyramiding was effective in conferring broad-spectrum resistance only when favorable genes were combined. The single genes *Co-4*^{2} and *Co-5* exhibited broad spectrum behavior. The effectiveness of gene pyramiding, however, would best be assessed under high disease pressure conditions over a period of time.

4.6 Recommendations

The bean populations developed should undergo further screening in bean anthracnose hotspot environments to have a deeper understanding of effectiveness of pyramided genes in respect to their broad-spectrum effect and durability. The pyramid gene combinations *Co-4^2+Co-5+Co-9* and *Co-4^2+Co-5* and the single genes *Co-4^2* and *Co-5* are recommended as the most effective resistance options against *C. lindemuthianum* in Uganda. The races 2047 and 767 are recommended for use in artificial inoculation while undertaking studies on resistance to bean anthracnose disease. Further studies should be conducted to develop and assess new pyramid combinations using other available anthracnose genes to discover other highly effective resistance combinations for pyramiding programs.

CHAPTER FIVE

Relationship between number of pyramided anthracnose resistance genes and yield traits among advanced common bean populations

Abstract

Pyramiding of resistance genes in commercial varieties would ensure reduction of yield losses resulting from the bean diseases. However, the effect and relationship between pyramided genes and plant yield traits is not well elucidated; and understanding genetic variability of breeding materials is crucial in ensuring and taking advantage of genetic gain among progenies. 53 $F_{4:5}$ and 69 $F_{4:6}$ advanced populations with varying number of resistance genes were evaluated in the field for yield performance. ANOVA was done to assess genetic variability of yield traits among the populations. Correlation and path coefficient analyses were done to uncover relationships between number of pyramided genes and the yield traits. ANOVA revealed significant variability for all yield traits among the populations and further revealed that PCV estimates were higher than GCV estimates. Broad sense heritability and genetic advance among populations were low for number of pods per plant, number of seeds per plant and seed weight per plant indicating importance of non-additive gene action. Number of pyramided genes showed a significant negative correlation with seed weight per plant (-0.17, $p<0.05$), number of pods per plant (-0.24, $p<0.05$) and number of seeds per plant (-0.19, $p<0.1$). Path coefficient analysis revealed a significant negative indirect effect plant (-0.25, $p<0.05$) of number of pyramided genes on seed weight per plant via number of seeds per. This suggests a possible reduction in yield that may be experienced as a result of pyramiding more resistance genes in a given genetic background. However, the significance of the potential yield loss may be reduced through integrating gene pyramiding with appropriate breeding strategies, which increase yield gain along with enhanced resistance to disease.

Key words: *Phaseolus vulgaris*, correlation analysis, path analysis, pyramided genes, yield

5.1 Introduction

Direct selection for yield may not be effective as it is a complex trait and depends upon the component traits. Understanding the association of traits, therefore, is crucial in developing an efficient breeding program that aims at developing high yielding varieties with effective resistance to bean anthracnose. Additionally, variability among germplasm is essential for the success of breeding programs for all economically important traits (Ramalho *et al.*, 1993). It is recommended that more than one trait be considered in a breeding program (Gonzalves *et al*., 2003). Correlation is useful in showing relationships among independent traits (Yucel *et al*., 2006). The estimated correlation measured enables the breeder to understand the changes that occur in a determined trait in function of the selection practiced on another correlated trait (Gonzalves *et al*., 2003). However, the quantification and interpretation of the magnitude of a correlation can result in errors when indirect selection strategy is used based on the effect of a third trait or of a group of other traits on them (Gonzalves *et al.,* 2003). Path coefficient analysis, therefore, further partitions the correlation coefficients into direct and indirect effects of a set of independent variables on the dependent variable (Guler *et al*., 2001). It also permits critical examination of the specific forces acting to produce a given correlation and measures the relative importance of each causal factor (Singh *et al*., 2013). Path coefficient analysis, therefore, plays an important role in determining the degree of relationship between a given trait and yield components (Ciftci *et al.,* 2004).

Gene pyramiding has been successfully used to pyramid resistance genes and create broad-spectrum and durable resistance (Ferreira *et al*., 2012). However, the relationship between pyramided genes and plant yield traits is not well elucidated. Previous studies indicated that gene pyramiding of resistance genes incurred no yield penalty on the plants. For example Liebenberg et al. (2005) successfully pyramided three bean rust resistance genes *Ur-3+, Ur-5, Ur-11* in advanced common bean lines and observed no yield reduction. Souza et al. (2014) also observed no yield penalty as a result of pyramiding rust resistance genes *Ur-5, Ur-11 and Ur-14 into advanced populations.*

There is compelling evidence, however, that disease resistance may affect crop performance (Heil, 2007). In order to establish resistance, plants have to cope with metabolic efforts that can cause considerable costs depending on plant growth stage and resource availability (Heil, 2007). The concept of fitness costs assumes that resistant plants have lower reproduction than

less resistant plants when compared under disease free conditions (Simms & Fritz, 1990). If resistance has a substantial cost, therefore, it has commercial significance because it may hinder the more important objective of increasing yield (Brown, 2002). Certain genes linked to a resistance gene may also affect yield and hence hamper the selection of commercially successful cultivars making it worthwhile for breeders to analyze such linkages and try to break them when the yield penalty is commercially significant (Brown, 2002). In other crops yield penalties due to enhanced resistance were reported. In wheat for instance, the *Lr34* resistance gene in combination with other *Lr* genes, is reported to provide effective resistance to brown rust but was associated with a 6% reduction in yield. This part of the study, therefore, was to establish the relationship between pyramided resistance genes and yield traits in advanced common bean populations.

5.2 Materials and methods

5.2.1 Field experimental set up and evaluation of advanced populations

Fifty three (53) $F_{4:5}$ and sixty nine (69) $F_{4:6}$ common bean families were developed using a cascading gene pyramiding scheme as indicated in Chapter Four, Section 4.2.2 and were selected for this study based on number of resistance genes inherited. The resistance genes for anthracnose included *Co-4*^{2}/*Co-4*^{3}, Co-*5* and *Co-9*. The $F_{4:5}$ and $F_{4:6}$ families were evaluated in the field for two seasons, using a nested randomized complete block design (RCBD) with three replicates. Single row plots of 1.5m length were used with a 50cm between row and 15cm within row spacing. The parents used in the breeding scheme were used as checks namely G2333, PI207262, RWR719, K132, NABE 4, NABE 13 and NABE 14. The trials were conducted at the National Agricultural Research Laboratories (NARL), Kawanda. Data was collected on plant vigor scored using a 1 – 5 scale where 1 = low vigor and 5 = high vigor; number of pods per plant, number of seeds per pod, pod length (cm), number of seeds per plant, seed weight per plant (gm), 100-seed weight (gm), days to 50% flowering, days to maturity.

5.2.2 Genetic variability and parameter estimation among populations

Analysis of variance (ANOVA) was carried out using GenStat discovery 12th edition (Anonymous, 2009) to partition variability for different characters. Genetic parameters to quantify genotypic variability among the genotypes for the characters under study were

estimated as follows: Genotypic variance (σ_g^2) = MSS $_{genotypes}$ – MSS $_{error}$ / Number of replications; Phenotypic variance (σ_p^2) = σ_g^2 + MSS $_{error}$; Genotypic coefficient of variability (GCV) = (σ_g/ μ) x 100; Phenotypic coefficient of variability (PCV) = (σ_p/ μ) x 100 (Wricke and Weber, 1986); Broad sense heritability (h_b^2) = σ_g^2/ σ_p^2 (Griffiths *et al.,* 2000); Genetic advance (GA) = h^2 x k x σ_p and Genetic advance as percent of mean (GAM) = GA/ μ x 100 (Johnson *et al.,* 1955).

Where; MSS $_{genotypes}$ = Mean Square for genotypes

MSS $_{error}$ = Mean Square for residual

μ = Grand mean for character

h_b^2 = Broad sense heritability

k = Standardized selection differential at 5% selection intensity = 2.063.

σ_p = Phenotypic standard deviation

σ_g = Genotypic standard deviation

GCV and PCV values were categorized as low (0 – 10%), moderate (10 – 20%) and high (20 % and above) according to Burton (1952). Heritability categorized according to Dabholker (1992) as low, (5 – 10%), medium (10 – 30%) and high (>30%). GA and GAM were categorized as low (<10%), moderate (10 – 20%) and high (>20%) according to Falconer and Mackay (1996).

5.2.3 Correlation analysis of number of pyramided genes and yield traits

The degree of association of different characters was done through correlation analysis with correlation coefficients calculated using the formula by Weher and Moorthy (1952). The standardization of the coefficients based on the standard deviations of the variables is the approach typically used to make coefficients comparable (Grace and Bollen, 2005). Therefore, data was standardized before correlation analysis.

$r_{xy} = Cov XY/\sqrt{\sigma_p^2 x.\sigma_g^2 y}$

Where; r_{xy} = Correlation between characters *x* and *y*;

Cov *XY* = Covariance between characters *x* and *y*

$\sigma_p^2 x.\sigma_g^2 y$ = Phenotypic variance of characters *x* and *y* respectively.

5.2.4 Path coefficient analysis of pyramided genes and yield traits

Path coefficient analysis was done according to the method described by Akintunde (2012) in order to identify yield traits via which number of pyramided genes directly or indirectly affected plant yield. Path coefficients were obtained by solving the simultaneous equations which express the basic relationships between correlations and path coefficients.

5.3 Results

5.3.1.1 Genetic variability among advanced common bean populations

The results for analysis of variance for the yield traits among advanced bean populations are presented in Table 5.1. Genotypes were highly significantly different (P<0.01 and P<0.05) for all the yield traits. Number of Seeds/plant contributed most to the total variation (61.9% for season 1 and 81.6% for season 2) while seeds per pod contributed the least (0.06%).

Table 5.1: Analysis of variance of the different yield traits

Traits	Sources of variation						
	MS Replication	MS Genotype	MS Error	F Ratio	% Contribution	Prob	C.V (%)
Season one							
100-seed wt	70.60	95.79	26.00	3.68***	2.29	<.001	18.2
Pod length	0.106	7.225	1.032	7.00***	0.17	<.001	11.1
Pods/ plant	18.64	146.59	99.22	1.48*	3.50	0.065	60.3
Seed wt/plant	104.6	239.7	119.0	2.01***	5.73	0.003	64.7
Seeds/ plant	197.0	2588.0	1,389	1.86***	61.87	0.008	62.2
Seeds/ pod	0.5526	2.5725	0.4895	5.26***	0.06	<.001	15.3
Days to 50% flow	-	9.137	5.167	1.77**	0.22	0.022	5.4
Days to maturity	-	9.945	5.667	1.76**	0.24	0.023	2.8
Plant height	-	1082.5	580.3	1.87**	25.88	0.014	39.2
Plant Vigor	-	1.2705	0.7484	1.70**	0.03	0.030	23.1
Season 2							
100-seed wt	156.17	39.95	10.07	3.97***	5.62	<.001	11.3
Seeds/ plant	214.1	580.0	400.0	1.45**	81.57	0.037	40.2
Pods/ plant	11.51	36.52	20.34	1.80***	5.14	0.002	35.0
Seed wt/ plant	11.87	46.92	26.07	1.80***	6.60	0.002	37.3
Plants at harvest	2.319	6.128	2.763	2.22***	0.86	<.001	22.7
Vigor	4.8667	1.5003	0.4018	3.73***	0.21	<.001	16.8

*** Highly significant (P<0.01), ** significant (P<0.05), * significant (P<0.1), MS = mean square, prob = probability, C.V% = Coefficient of variation

During season one seed weight per plant had the highest coefficient of variation (64.7%) followed by number of seeds per plant (62.2%), pods per plant (60.3%) and plant height (39.2%), while during season two number of seeds per plant had the highest coefficient of variation (40.2%) followed by seeds weight per plant (37.3%) and pods per plant (35.0%). Hence variability in the advanced populations was considered for further analysis.

5.3.1.2 Phenotypic and Genotypic coefficient of variability among populations

Parameter estimates from the segregating $F_{4:5}$ and $F_{4:6}$ bean populations are presented in Table 5.2.

Table 5.2: Parameter estimation among advanced bean populations

	Genetic Parameters							
Characters	**Grand Mean (μ)**	**GV**	**PV**	**GCV**	**PCV**	**h_b^2**	**GA**	**GAM**
Season one (F_5)								
Pod length	9.1	2.1	3.1	15.7	19.3	68	2.5	27.5
Seeds/ pod	4.6	0.7	1.2	18.2	23.8	58	1.4	30.4
Pods/ plant	16.5	15.8	115.0	24.1	65.0	14	3.0	18.2
Seeds/ plant	59.9	399.7	1788.7	33.4	70.6	22	17.4	29.1
Seed weight/ plant	16.9	40.2	159.2	37.5	74.6	25	7.8	46.2
100-seed weight	28.0	23.3	49.3	17.2	25.1	47	7.2	25.7
Days to 50% flow	42.5	4.0	9.1	4.7	7.1	44	2.7	6.4
Days to maturity	84.1	4.3	10.0	2.5	3.8	43	2.8	3.3
Plant height	61.4	502.0	1083.0	36.5	53.6	46	31.4	51.1
Plant Vigor	3.7	0.5	1.3	19.1	30.5	38	0.9	24.3
Season two (F_6)								
Plant Vigor	3.8	0.4	0.8	16.0	23.1	50	0.9	23.7
Pods/plant	12.9	5.4	25.7	18.0	39.4	21	2.1	16.3
seeds/plant	49.9	60.0	460.0	15.5	43.0	13	4.4	8.8
Seed wt/plant	13.7	7.0	33.0	19.2	41.8	21	2.4	17.5
100-seed wt	27.9	10.0	20.0	11.3	16.1	50	4.6	16.5

GV = Genetic variance, PV = Phenotypic variance, GCV = Genetic coefficient of variability, PCV = Phenotypic coefficient of variability, GA = Genetic advance, GAM = genetic advance as a percentage of the grand mean, h_b^2 = broad sense heritability (%)

For all characters PCV estimates were higher than GCV. In season one, days to 50% flowering and days to maturity had low PCV values (<10%). Pod length had a moderate PCV value (10 – 20%). Seeds per pod, 100-seed weight, plant vigor, pods per plant, seeds per plant, seed weight per plant and plant height had high PCV values (>20%). In season two, 100-seed weight had moderate PCV values while all the other traits had high PCV values (>20%). In season one, days to 50% flowering and days to maturity had low GCV values (0 –

10%). Pod length, seeds per pod, 100-seed weight and plant vigor had moderate GCV values (10 – 20%). Pods per plant, seeds per plant, seed weight per plant and plant height had high GCV values (>20 %).

5.3.1.3 Heritability and genetic advance among advanced bean populations

Pods per plant, seeds per plant and seed weight per plant had low to moderate broad sense heritability values (10% – 30%) while, pod length, seeds per pod, 100-seed weight, days to 50% flowering, days to maturity, plant height and plant vigor had high heritability values (>30%). Pod length had the highest broad sense heritability of 70% followed by seeds per pod (60%), 100-seed weight (50%), plant vigor (50%), while pods per plant (10%) and seeds per plant (10%) had the lowest heritability estimates.

Pod length, seeds per pod, 100-seed weight, plant height and plant vigor had high heritability estimates (>30%) and high Genetic advance as percent of mean (GAM) (>20%). Seeds per plant and seed weight per plant had moderate heritability estimates (10 – 30%) and high GAM. Pods per plant had a low heritability (5 – 10%) and a moderate GAM. However, days to 50% flowering and days to maturity had high heritability values but low GAM.

5.3.2 Yield traits performance among advanced bean populations

Advanced populations, number of inherited resistance genes and the performance of different yield traits are presented in Table 5.3.

Table 5.3: F_5 Advanced bean populations and their yield performance

Family code	SWP	NPG	PV	PH	DF	DM	SP	PL	NPP	NSP	100SW
A16.1.2.1.6.3	26.2	0	4.0	55.8	40.0	83.5	3.2	9.4	26.3	60.0	40.8
A16.1.2.1.6.2	7.1	0	3.0	26.7	40.0	83.0	2.3	9.1	15.5	31.0	29.6
B44.7.5.8.112.2	15.9	0	4.5	33.5	42.0	81.5	5.3	8.8	18.3	58.5	32.4
B44.7.5.8.112.1	6.8	0	3.5	36.5	44.0	81.0	3.4	7.2	7.3	27.7	22.7
C44.1.6.7.130.1	22.8	0	3.0	85.6	45.7	85.7	5.7	10.0	26.5	98.0	24.2
C44.1.6.7.130.2	18.7	0	3.0	93.8	46.3	85.3	6.1	10.5	16.3	73.0	25.6
C44.1.6.7.130.3	24.5	0	3.3	65.2	46.0	89.0	5.3	10.2	24.8	96.5	25.5
D16.3.3.2.152.1.2	9.0	0	2.0	46.3	44.0	83.5	5.3	11.1	10.2	43.8	19.1
D16.3.3.2.152.2.1.3	12.7	0	3.0	72.0	44.0	85.0	8.0	12.5	10.0	49.0	25.9
A16.1.2.3.7.1	45.8	1	4.5	59.7	41.0	83.0	5.5	10.9	38.8	155.2	30.1
A16.1.2.3.7.2	24.3	1	3.0	65.0	42.0	84.0	3.6	8.8	26.8	87.5	33.5
A16.6.1.6.25.1	12.6	1	3.3	46.2	39.3	80.7	4.7	8.6	14.4	51.6	22.2
A44.5.7.1.1.1	4.3	1	4.0	19.3	38.0	80.0	2.6	6.2	9.6	21.1	22.8
B44.7.8.2.91.1	15.6	1	4.0	77.3	42.3	85.0	4.0	8.4	19.3	54.9	31.3
B44.7.8.2.91.10	22.0	1	4.3	88.3	38.0	83.3	5.4	10.1	19.1	77.7	27.8

B44.7.8.3.92.3	17.0	1	4.5	57.0	40.0	83.3	4.1	8.8	17.9	56.3	30.3
B44.7.8.2.91.11	31.6	1	5.0	77.7	42.0	85.0	6.0	11.9	16.8	89.0	31.2
B44.7.8.3.92.2.3.4	11.5	1	4.0	54.0	43.0	82.0	4.5	9.5	11.8	32.3	33.9
A44.5.2.1.26.2	12.2	2	3.5	24.2	40.0	80.0	4.4	7.9	14.0	43.4	21.4
A44.5.2.3.28.1	9.8	2	3.3	99.2	42.0	85.0	4.9	8.7	12.3	49.6	19.7
A44.5.2.1.26.1	4.1	2	3.0	21.3	38.0	80.0	3.2	6.5	7.5	19.5	20.9
A44.5.1.11.46.1	8.7	2	2.0	57.0	43.3	85.0	4.8	9.1	18.3	59.0	14.3
B89.5.2.10.118.15	4.3	2	2.5	28.0	44.0	85.0	3.4	6.6	7.0	23.7	21.1
B89.5.1.1.81.2	10.4	2	4.3	58.8	43.3	85.0	5.6	10.3	11.4	54.0	18.7
B89.5.1.1.81.3	7.6	2	4.0	52.7	44.0	84.5	5.3	10.2	7.9	32.6	24.2
B89.5.2.7.117.1.1	10.4	2	3.0	43.0	44.0	85.0	3.0	6.5	17.0	46.0	22.5
B89.5.2.7.117.2.2	1.3	2	2.0	17.5	45.0	85.0	1.7	2.5	1.0	5.0	26.6
B44.7.9.5.99.2.3.4	11.7	2	3.5	83.7	45.0	85.5	5.0	8.8	14.0	47.7	25.7
B89.5.1.1.81.1.1	7.8	2	3.0	71.0	45.0	84.0	5.3	9.7	11.0	37.0	21.2
C16.1.3.8.136.1	17.2	2	4.0	59.2	42.0	82.5	4.9	9.9	16.8	52.9	32.2
C16.1.3.8.136.2	26.8	2	5.0	77.7	44.3	88.7	5.1	10.3	17.8	72.2	34.7
C44.1.4.5.142.1	23.9	2	4.0	68.3	46.0	88.3	4.8	9.3	22.3	85.2	28.3
C44.1.4.5.142.3	14.0	2	4.0	48.6	43.7	83.7	3.6	7.7	12.6	44.3	30.2
C44.1.4.5.142.4	27.8	2	4.0	50.5	40.0	84.5	4.5	8.8	26.5	96.0	29.2
C44.1.4.7.143.1	20.6	2	4.0	94.2	43.5	85.0	4.5	8.6	18.1	70.5	28.4
D16.3.3.1.151.1	15.3	2	3.5	33.8	42.0	83.3	5.3	11.9	17.5	52.8	29.5
A44.5.9.1.14.2	2.3	3	2.0	14.0	43.0	83.5	2.3	4.8	2.5	7.5	32.6
A44.5.2.3.28.2	11.0	3	3.3	86.9	42.3	84.7	4.1	7.7	12.6	47.9	20.2
A44.5.2.3.28.4	13.5	3	2.7	73.7	43.0	83.3	4.4	8.3	16.2	61.9	21.8
A44.5.9.1.14.1	13.6	3	3.0	58.0	38.0	82.0	4.2	8.6	13.5	42.0	32.5
C44.1.4.3.141.1	21.9	3	4.7	83.4	41.7	86.7	4.0	7.6	21.7	73.2	31.1
C44.1.4.3.141.2	12.1	3	4.5	53.3	40.0	83.0	3.0	6.4	11.6	37.6	33.2
D16.3.3.8.157.3	17.3	3	4.3	36.1	40.7	81.7	4.8	9.7	12.6	47.8	36.2
D16.3.3.9.158.1	16.3	3	4.0	38.8	40.7	81.0	4.8	10.3	15.3	49.9	32.3
D16.3.3.10.159.6	20.0	3	4.0	68.0	42.0	84.0	5.3	11.8	15.7	67.7	29.1
D16.3.3.11.160.2	23.4	3	4.7	98.4	42.0	83.0	6.0	11.5	14.9	68.2	35.1
D42.12.4.1.161	14.3	3	5.0	94.0	43.0	82.0	3.4	8.1	16.7	46.5	31.3
D16.3.3.11.160.5.6	28.6	3	5.0	69.0	42.0	83.0	6.1	11.7	19.0	84.2	35.0
D16.3.3.11.160.5.8.3	22.7	3	4.0	57.0	44.0	83.0	4.3	11.3	17.0	71.0	32.0
D16.3.3.11.160.5.8	11.1	3	4.0	110.5	44.0	85.0	6.0	12.0	13.0	44.0	25.1
B44.7.9.8.102.1	8.4	4	3.5	56.7	43.5	87.0	4.4	9.5	13.3	29.8	27.9
B44.7.2.2.76.4	7.9	4	3.5	23.4	42.0	85.0	4.0	7.9	7.0	42.5	19.7
B44.7.2.2.76.1.1	1.8	4	4.0	34.0	42.0	87.0	3.0	6.3	2.0	11.0	16.4
Std deviation	**8.7**	**1.2**	**0.8**	**24.2**	**2.1**	**2.1**	**1.2**	**2.0**	**6.8**	**26.5**	**5.7**
Parents											
K132	36.1	0	3.3	22.2	41.4	80.0	4.0	11.7	24.0	86.0	42.0
NABE 4	19.4	0	4.5	30.0	40.7	81.3	3.6	8.5	13.3	46.5	41.2
NABE 13	22.2	1	4.6	28.8	38.8	81.8	4.8	10.0	13.0	58.4	36.5
NABE 14	14.1	1	5.0	25.9	39.3	82.7	4.6	11.4	9.1	38.4	36.2
RWR719	34.3	1	3.8	55.5	43.5	84.0	7.0	10.8	27.7	161.3	21.1
PI207262	19.8	2	4.6	70.4	38.2	81.0	5.5	7.5	26.1	99.3	20.2
G2333	37.1	3	4.4	243.0	44.5	90.0	8.2	11.2	23.5	196.9	19.2
Std deviation	**9.4**	**1.1**	**0.6**	**79.2**	**2.4**	**3.3**	**1.7**	**1.6**	**7.5**	**60.1**	**10.3**

A = pedigree 12x8xRWR719XK132; B = pedigree 12x8xRWR719xNABE4; C = pedigree 12x8xRWR719xNABE13; D = pedigree 12x8xRWR719xNABE14; SWP = Seed weight per plant; NPG = Number of pyramided genes; PV = Plant vigor; PH = Plant height; DF = Days to flowering; DM = Days to maturity; SP = Seeds per pod; PL = Pod length; NPP = Number of pods per plant; NSP = Number of seeds per plant; 100SW = 100-seed weight.

The overall best five (5) lines/ genotypes in terms of seed weight per plant were line 16.1.2.3.7.1 (12x8xRWR719xK132) (45.8gms) with single inherited gene, donor parent G2333 (37.1gm), parent K132 (36.1gm), parent RWR719 (34.3gm) and line 44.7.8.2.91.11 (12x8xRWR719xNABE4) (31.6gm) with a single inherited gene. Three lines with a four-gene pyramid namely 44.7.9.8.102.1 (12x8xRWR719xNABE4) (8.4gm), 44.7.2.2.76.4 (12x8xRWR719xNABE4) (7.9gm) and 44.7.2.2.76.1 (12x8xRWR719xNABE4) (1.8gm) had a yield range of 1.8gm – 8.4gm per plant. Fourteen genotypes with a three-gene pyramid had a yield range of 11.0gm and 28.4gm. Eighteen genotypes with a two-gene pyramid had a yield range of 1.8gm and 27.8gm; nine genotypes with a single gene had a yield range of 4.3gm and 45.8gm; and nine genotypes with no-gene had a yield range of 6.8 – 26.2gms. Among the pyramided lines, line 16.3.3.11.160.5.6 (12x8xRWR719xNABE14) with a three-gene pyramid performed best in terms of yield per plant (28.6gm) followed by 44.1.4.5.142.4 (12x8xRWR719xNABE13) (27.8gm) and 16.1.3.8.136.2 (12x8xRWR719xNABE13) (26.8gms) both with a two-gene pyramid. These three lines were among the overall best ten genotypes in terms of seed weight per plant. In terms of seed yield per plant, populations derived from cultivar NABE 13 performed best with an average yield of 20.9gms followed by NABE 14 derived populations (17.3gms), while NABE 4 derived populations performed least (11.3gms) (Table 5.4). Among the recurrent parents K132 performed best (36.1) followed by NABE 13 (22.2gms), NABE 4 (19.4gms) and NABE 14 (14.1). Derived populations on average yielded less than their respective parents except those of NABE 14 (17.3gms).

Table 5.4: Comparison of performance between parents and their derived advanced populations/ lines

Genotypes	SWP (gms)	PV	SP	NPP	NSP	100SW (gms)
K132 Parent	36.1	3.3	4.0	24.0	86.0	42.0
Kl32 derived populations	14.0	3.2	3.9	16.3	52.7	25.9
NABE 4 parent	19.4	4.5	3.6	13.3	46.5	41.2
Nabe 4 derived populations	11.3	3.7	4.3	11.9	42.7	25.5
NABE 13 parent	22.2	4.6	4.8	13.0	58.4	36.5
Nabe 13 derived populations	20.9	4.0	4.7	19.5	72.7	29.3
NABE 14 parent	14.1	5.0	4.6	9.1	38.4	36.2
Nabe 14 derived populations	17.3	4.0	5.4	14.7	56.8	30.1

SWP = Seed weight per plant; PV = Plant vigor; SP = Seeds per pod; NPP = Number of pods per plant; NSP = Number of seeds per plant; 100SW = 100-seed weight

There was a general decline, however, of 100-seed weight among derived populations for all the four recurrent parents. Declining trends in seed weight per plant (Figure 5.1) and number of pods per plant (Figure 5.3) were observed as number of pyramided genes increased.

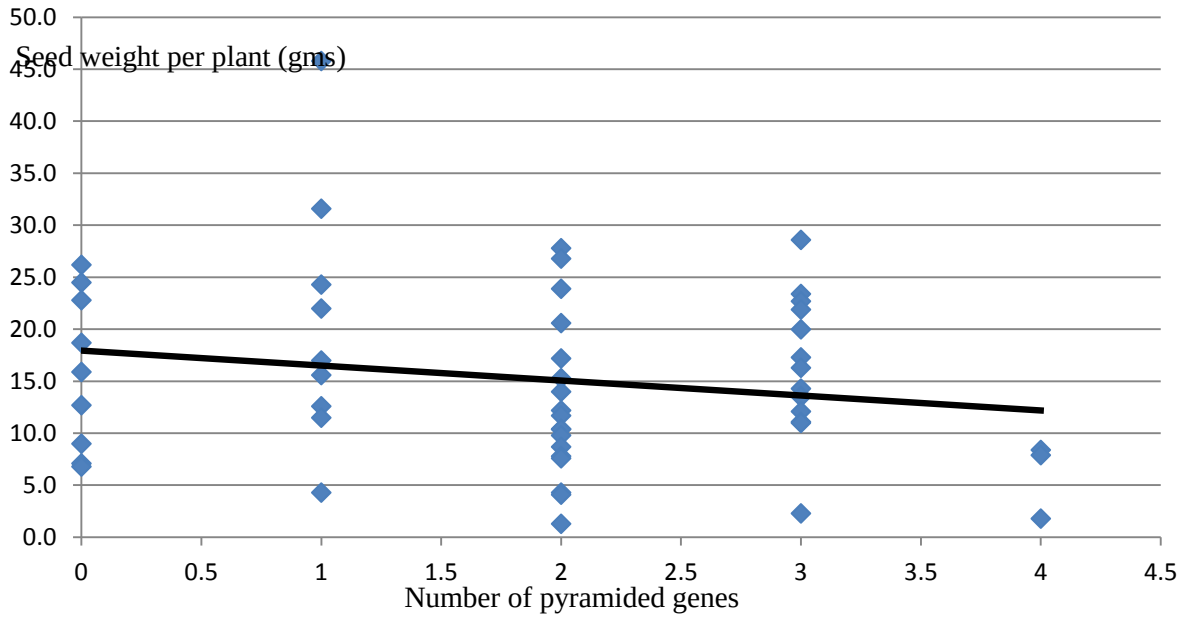

Figure 5.1: Trend of Seed weight per plant (SWP) with increasing number of pyramided genes

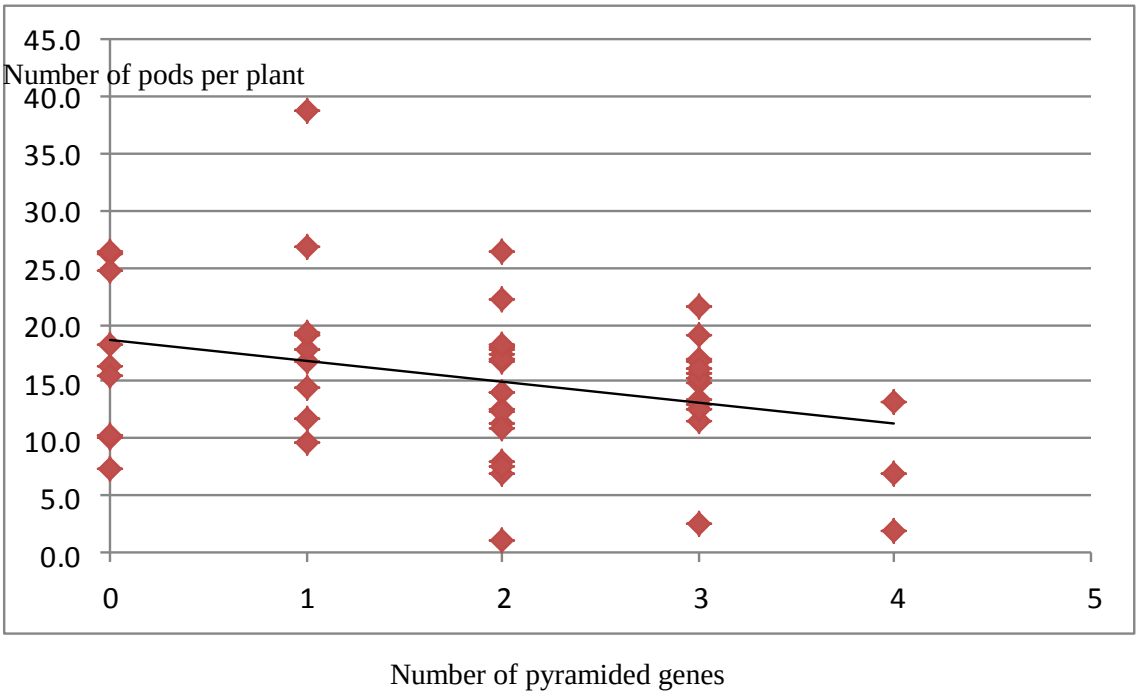

Figure 5.2: Trend of Number of pods per plant (NPP) with increasing number of pyramided genes

5.3.3 Correlation between number of pyramided genes and yield associated traits

The results of correlation between number of pyramided genes (NPG) and yield traits of the advanced bean families are presented in Table 5.5. A highly significant negative correlation was observed between number of pyramided genes and seed weight per plant (-0.17, *p<0.01*), while significant negative correlations were observed between number of pyramided genes and number of pods per plant (-0.24, $p<0.05$) and number of seeds per plant (-0.19, $p<0.1$).

Table 5.5: Correlation matrix for seed weight per plant and different plant growth and yield characters

Variables	SWP	NPG	PV	PH	DF	DM	NSP	PL	NPP	NSPT	100 SW
SWP	1.00										
NPG	-0.17***	1.00									
PV	0.56***	-0.02ns	1.00								
PH	0.45***	0.00	0.33***	1.00							
DF	-0.03ns	-0.09ns	-0.24**	0.30**	1.00						
DM	0.17ns	0.08ns	-0.03ns	0.39***	0.63***	1.00					
NSP	0.46***	-0.18ns	0.25**	0.56***	0.25**	0.18ns	1.00				
PL	0.56***	-0.17ns	0.36***	0.50***	0.13ns	0.11ns	0.86***	1.00			
NPP	0.82***	-0.24**	0.39***	0.40***	-0.05ns	0.15ns	0.31**	0.45	1.00		
NSPT	0.92***	-0.19*	0.39***	0.49***	0.07ns	0.25**	0.52***	0.56***	0.85***	1.00	
100SW	0.42***	-0.02ns	0.42***	0.12ns	-0.19*	-0.12ns	0.01ns	0.23*	0.03ns	-0.09ns	1.00

*** Highly significant (P<0.01), ** Significant (P<0.05), * Significant (P<0.1). ns = not significant. *SWP*= seed weight per plant, NPG = number of pyramided genes, PV= plant vigor, PH= plant height, DF = days to 50% flowering, DM = days to maturity, NSP = number of seeds per pod, PL = pod length, NPP = number of pods per plant, NSPT = number of seeds per plant and 100SW = 100-seed weight.

5.3.4 Path coefficient analysis of number of pyramided genes and yield traits

The results of estimates of direct and indirect path coefficients using seed weight per plant as the response variable are presented in Table 5.6.

Table 5.6: Direct and indirect path coefficients for seed weight per plant

Variables	NPG	PV	PH	DF	DM	NSP	PL	NPP	NSPLT	100SW	SWP (r)
NPG	**-0.012**	0.013	-0.000	0.004	0.002	0.012	-0.011	0.050	-0.246**	-0.006	**-0.194***
PV	-0.002	**0.082**	-0.006	0.010	-0.001	-0.017	0.022	-0.052	0.371***	0.143	**0.549*****
PH	-0.000	0.027	**-0.018**	-0.012	0.010	-0.038	0.031	-0.063	0.478***	0.033	**0.447*****
DF	0.001	-0.020	-0.005	**-0.040**	0.016	-0.017	0.008	0.008	0.070	-0.054	**-0.034**
DM	-0.001	-0.002	-0.007	-0.025	**0.025**	-0.012	0.007	-0.024	0.240**	-0.034	**0.165**
NSP	0.002	0.021	-0.010	-0.010	0.005	**-0.067**	0.053	-0.048	0.511***	0.002	**0.456*****
PL	0.002	0.030	-0.009	-0.005	0.003	-0.058	**0.061**	-0.070	0.548***	0.063	**0.565*****
NPP	0.002	0.026	-0.007	0.002	0.004	-0.021	0.027	**-0.157**	0.882***	0.105	**0.867*****
NSPLT	0.003	0.031	-0.009	-0.003	0.006	-0.035	0.035	-0.142	**0.975*****	0.076	**0.937*****

100SW	0.000	0.042	-0.002	0.008	-0.003	-0.000	0.014	-0.060	0.268**	**<u>0.276**</u>**	**0.544*** **

*** Highly significant (P<0.01), ** Significant (P<0.05), * Significant (P<0.1). SWP (r) = seed weight per plant, NPG = number of pyramided genes, PV = plant vigor, PH = plant height, DF = days to 50% flowering, DM = days to maturity, NSP = number of seeds per pod, PL = pod length, NPP = number of pods per plant, NSPLT = number of seeds per plant and 100SW = 100-seed weight. The sum of direct and indirect path coefficients is bolded in the last column. Direct path coefficients appear diagonally in bold and underlined. The rest of the coefficients are indirect coefficients.

Number of pyramided genes (NPG) had a negative but not significant (-0.012) direct correlation with seed weight per plant. However, a significant (P<0.05) negative indirect effect of number of pyramided genes on seed weight per plant was observed via number of seeds per plant (-0.25).

5.4 Discussion

5.4.1 Genetic variability among the advanced populations

The success of genetic advance under selection depends on three factors namely genetic variability, heritability and selection intensity (Allard, 1960). In this study, ANOVA revealed significant variations for all yield traits among the advanced populations indicating a significant amount of variability among the genotypes for all traits. All traits except days to 50% flowering and days to maturity had moderate to high PCV and GCV estimates implying a high phenotypic and genotypic variability for all traits except days to 50% flowering and days to maturity. For all traits PCV estimates were higher than GCV estimates implying that environmental effects are important in the expression and improvement of these traits. The results of high PCV and GCV values for number of pods per plant number of seeds per plant and seed weight per plant indicate a high variability of these yield traits. These findings are in agreement with Singh et al. (1994), Raffi and Nath (2004) and Negash (2006). Plant breeders ascribe genetic improvement in any crop plant to the amount of variability present in the germplasm. The low PCV and GCV of days to 50% flowering and days to maturity suggests a low response to selection for these traits as compared to number of pods per plant, number of seeds per plant and seed weight per plant, which had the highest estimates.

Heritability is a genetic effect of a phenotypic appearance that can be passed on from the parents to the progeny. The high broad sense heritability estimates observed for pod length, 100-seed weight, 50% flowering, days to maturity, plant vigor, plant height, pod length and seeds per pod indicate that these traits could be improved through selection because of a close correspondence between genotype and phenotype. Low to moderate heritability estimates

were observed for pods per plant, seeds per plant and seed weight per plant indicating a low correspondence between genotype and phenotype and that these yield traits are highly influenced by environment (Singh, 2009). Therefore, genetic improvement through selection would be difficult. However, high broad sense heritability is not always associated with high genetic advance (Amin *et al*., 1992) and relying on it alone may not be effective in ensuring genetic gain through selection. Narrow sense heritability (h^2_n), which is a ratio of the additive variance to the total phenotypic variance, is a more useful parameter in the selection process in plant breeding. However, in absence of narrow sense heritability estimates, Johnson et al. (1955) suggested the use of broad sense heritability estimates in conjunction with predicted genetic advance for effective selection.

High heritability estimates (>30%) and high Genetic advance as percent of mean (GAM) (>20%) were observed for pod length, number of seeds per pod, 100-seed weight, plant height and plant vigor implying that these yield traits respond well to selection (Singh, 2009) and that the genotype is closely associated with the observed phenotype (Govindaraj *et al*., 2011). High heritability but low to moderate GAM estimates were observed for days to 50% flowering, days to maturity and number of pods per plant implying low response to selection (Govindaraj *et al*., 2011) and the need to explore other breeding methods for their improvement. According to Singh (2009), Brown and Caligari (2011), and Kute and Kumar (2013), high genetic advance indicates that the trait is governed by additive genes and selection is beneficial for such a trait; while low genetic advance indicates that the trait is governed by non-additive genes and heterosis breeding may be beneficial.

5.4.2 Relationship between number of pyramided genes and yield

Among families with pyramided genes the family 16.3.3.11.160.5.6 (12x8xRWR719xNABE14) with a three-gene pyramid performed best in terms of seed weight per plant (28.6gm) followed by 44.1.4.5.142.4 (12x8xRWR719xNABE13) (27.8gm) and 16.1.3.8.136.2 (12x8xRWR719xNABE13) (26.8gms) both with a two-gene pyramid. These three lines were among the overall top ten genotypes in terms of seed weight per plant. These results indicate that with effective selection strategies and a deeper understanding of genetic parameters and variability of the populations, it is possible to simultaneously breed for high yield and high number of pyramided genes.

Number of pyramided genes had a significant negative correlation with seed weight per plant (-0.17), number of pods per plant (-0.24, $p<0.05$) and number of seeds per plant (-0.19,

p<0.1). This indicates that breeding for a higher number of pyramided genes may negatively affect yield through reduction of seed weight per plant, number of pods and number of seeds per plant. The reduction in seed weight per plant was also evident in the reduction of 100-seed weight observed when recurrent parents K132, NABE 4, NABE 13 and NABE 14 were compared with their respective derived populations with pyramided genes (Table 5.4).

Further analysis through path coefficient analysis revealed that a significant (*P*<0.05) negative indirect effect of number of pyramided genes on seed weight per plant which occurred via number of seeds per plant (-0.25), further implying a possible yield cost resulting from pyramiding a higher number of resistance genes. Though common in other crops like wheat, yield penalties as a result of pyramided resistance genes are not commonly reported in common beans. Wahome et al. (2011) observed that advanced bush snap bean lines with pyramided resistance genes to angular leaf spot, anthracnose and rust failed to meet the yield and quality expectations of commercial varieties.

Contrary to the negative relationship observed in this study between number of pyramided genes and yield, a number of studies such as Coyne et al. (2000), Liebenberg et al. (2005), Fininsa and Tefera (2007), Ragagnin et al. (2009), Souza et al. (2014), Mulanya et al. (2014) and Miklas et al. (2017) reported no yield penalty as a result of pyramiding resistance genes in common beans. It should be noted, however, that these studies focused on the evaluation of pyramided lines in comparison with available commercial varieties included as checks, but did not investigate the relationship between yield and pyramided genes. This study evaluated yield of lines with no-gene, single gene and pyramided genes in comparison with commercial but susceptible varieties K132, NABE 4, NABE 13 and NABE14 and results could not clearly reveal a yield penalty, given that three lines with two-gene and three-gene pyramided genes were among the best 10 performing out of over 50 advanced lines evaluated (Table 5.3). However, declining trends of seed weight per plant (Figure 5.2) and number of pods per plant (Figure 5.3) were observed with increasing number of pyramided genes. Further correlation and path analyses revealed significant negative correlations between number of pyramided genes and yield traits (seed weight per plant, number of pods per plant and number of seeds); and a significant negative indirect effect with seed weight per plant via number of seeds per plant. However, the potential yield cost incurred may lose its significance when weighed against the potential benefit of increased broad-spectrum and durable resistance against diverse *C. lindemuthianum* races.

The low to moderate heritability (0.2-0.3) observed with pods per plant, number of seeds per plant and seed weight per plant indicates these yield traits are influenced by environment and controlled by non-additive genes making improvement by selection difficult. Both additive and non-additive gene effects are reported to control yield traits (Gonzalves et al., 2008) making heterotic breeding a viable strategy to enhance yield and limit possible negative effects on yield that could arise due to resistance gene pyramiding.

5.5 Conclusion

Number of pyramided resistance genes negatively influenced seed weight per plant, number of pods per plant and number of seeds per plant; and further negatively affected seed yield through an indirect effect on seed weight per plant via number of seeds per plant; implying a possible negative effect of pyramided genes on yield. The high phenotypic and genotypic variability observed for the yield traits shows that there is a high potential among the populations for developing superior genotypes/ lines with higher yields and enhanced broad-spectrum resistance to bean anthracnose.

5.6 Recommendations

The pyramided lines 16.3.3.11.160.5.6 (12x8xRWR719xNABE14), 44.1.4.5.142.4 (12x8xRWR719xNABE13) and 16.1.3.8.136.2 (12x8xRWR719xNABE13) are recommended for further development and use as elite breeding material or for national evaluation and release. Further studies should be conducted to investigate specific (SCA) and general (GCA) of common bean cultivars to those higher breeding potentials for use as founding parents so as to maximize yield gain and reduce possible negative yield effects in gene pyramiding programs.

CHAPTER SIX

General overview

The study was undertaken with the overall objective to contribute to the understanding of gene pyramiding for broad-spectrum resistance to bean anthracnose disease in Uganda.

The study investigated the pathogenic variation of *Colletotrichum lindemuthianum* in Uganda through screening of differential cultivars with isolates from eight districts. It was found that; *C. lindemuthianum* diversity was high with 27 races characterized from 51 isolates; races 2047 and 4095 were the most widely distributed in the sampled regions and races 4095, 2479 and 2047 respectively were the most virulent with race 4095 causing a susceptible reaction on all the 12 differentials. The pathogenic variability of *C. lindemuthianum* was highest in the Eastern and South Western highland regions of Uganda implying the need to deploy pyramided genes in these regions for effective management of bean anthracnose. The cultivars G2333, Cornell, TU, and AB136 showed the most broad-spectrum resistance to the 27 races implying that they are best suited as sources of effective resistance in breeding programs in Uganda.

The study undertook a gene pyramiding scheme for bean anthracnose resistance genes with the help of SCAR markers and assessed the effectiveness of both single and pyramided genes. The findings revealed that; the SCAR markers *SBB14, SAS13, SAB3* and *SB12* could be successfully employed to tag and pyramid *Co-4*^{2}/*Co-4*^{3}, *Co-5* and *Co-9* anthracnose resistance genes; and as a result 38 populations with two anthracnose resistance genes; nine (9) populations with three anthracnose resistance pyramided genes; and 25 populations with single anthracnose genes were successfully identified. The broad-spectrum resistance against *C. lindemuthianum* conferred by different gene pyramids differed in effectiveness with *Co-4*^{2}*+Co-5+Co-9* and *Co-4*^{2}*+Co-5* combinations emerging as the best. The effectiveness of single genes against *C. lindemuthianum* also varied with *Co-4*^{2} and *Co-5* single genes emerging the best and with good complementarity with each other. However, *Co-9* was antagonistic with other genes and was always associated with increased symptoms.

Analysis of genetic variability among advanced populations and assessing the relationship between number of pyramided genes and yield traits revealed; a high ($P<0.01$, $P<0.05$)

genetic variability for all yield traits in the bean populations indicating high potential for genetic improvement; PCV estimates higher than GCV estimates implying the importance of environment in the improvement; low to moderate heritability estimates for number of pods per plant, number of seeds per plant and seed weight per plant; and low to moderate GAM estimates for days to 50% flowering and days to maturity and number of pods per plant implying that these traits are controlled by non-additive genes effects, respond poorly to selection and therefore heterosis breeding is beneficial for their improvement; number of pyramided genes had a negative relationship with yield traits such as seed weight per plant, number of pods per plant and number of seeds per plant; and also had a significant negative indirect effect with seed weight per plant via number of seeds per plant implying a possible yield penalty; The advanced lines 16.3.3.11.160.5.6, 44.1.4.5.142.4 and 16.1.3.8.136.2 with pyramided genes were among the top 10 performing lines.

6.1 General conclusions

Pathogenic variation of *C. lindemuthianum* was high with 27 races some of which are potentially new and highly aggressive raising the need for enhanced efforts towards management and control of bean anthracnose disease in Uganda. Resistance gene pyramiding was achieved using SCAR markers and was effective in conferring broad-spectrum resistance with the pyramids *Co-4*^{2}*+Co-5+Co-9* and *Co-4*^{2}*+Co*-5 emerging as the best pyramid options against *C. lindemuthianum* in Uganda. The single genes *Co-4*^{2} and *Co-5* were the most effective indicating that single genes also possess broad-spectrum quality of resistance, while the single gene *Co-9* showed antagonism and should be avoided for deployment in Uganda. A negative relationship observed between number of pyramided genes and yield traits such as seed weight per plant and number of pods per plant indicates a possible yield penalty as a result of gene pyramiding. However, possible yield cost could be minimized through enhancing yield gain by integrating heterosis breeding in the pyramiding program.

6.2 General recommendations

The Cultivars G2333, Cornell 49-242, TU, AB136 and the single genes *Co-4*^{2}, *Co-5*^{2}, *Co-5*, *Co-1*^{3}, *Co-1*^{5}, *Co-3*, *Co-6*, *co-8* are recommended as sources of broad-spectrum resistance against *C. lindemuthianum* in Uganda. The pyramid *Co-4*^{2}*+Co-5+Co-9* and *Co-4*^{2}*+Co-5* and the single genes *Co-4*^{2} and *Co-5* are recommended for deployment against *C. lindemuthianum* in Uganda. The pyramided lines 16.3.3.11.160.5.6 (12x8xRWR719xNABE14), 44.1.4.5.142.4 (12x8xRWR719xNABE13) and 16.1.3.8.136.2

(12x8xRWR719xNABE13) are recommended for further development and use as elite breeding material or for national evaluation and release. The advanced populations should undergo further field screening to assess for durability of resistance. New sources of resistance and effective pyramid combinations should be identified. Further studies should be conducted to identify parents with specific (SCA) and general (GCA) combining for use as founding parents in gene pyramiding programs so as to enhance yield gain and counteract any possible negative effects that may arise as a result of gene pyramiding.